A Randomized Trial of Neprilysin Inhibition with Sacubitril/valsartan vs Irbesartan in Chronic Kidney Disease

by

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Thesis Declarations

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by me and has not been submitted in any previous application for any degree, at the University of Warwick or at any other Institution.

The work presented in this thesis was compiled by me except in the cases outlined below:

Chapter 6: The original trial Protocol, upon which the methodology described in this thesis is based, was devised by the Chief Investigator of the trial, Professor Richard Haynes, and the two Principal Investigators, Professor Colin Baigent and Professor Martin Landray. I developed subsequent protocol amendments during the trial. The Trial Steering Committee, of which I was a member, provided comments and approval of the final protocol before initiation of the UK HARP-III trial.

The statistical methods described in this thesis are based on trial Data Analysis Plan for UK HARP-III. I worked with other clinicians, Professor Richard Haynes and Dr William Herrington, and the trial statistician, Dr Natalie Staplin, to draft the Data Analysis Plan.

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Publications containing material from this thesis

Parts of this thesis contains material that has previously been published as outlined below:

- **Judge P**, Haynes R, Landray MJ, Baigent C. Neprilysin inhibition in chronic kidney disease. *Nephrol Dial Transplant*. 2015;30(5):738-43
  Chapters 1, 2, 3, 4 and 8
- **Judge PK**, Haynes R, Herrington WG, Storey BC, Staplin N, Bethel A, Bowman L, Brunskill N, Cockwell P, Dayanandran R, Hill M, Kalra PA, McMurray JJ, Taal M, Wheeler DC, Landray MJ, Baigent C. Randomized multicentre pilot study of sacubitril/valsartan versus irbesartan in patients with chronic kidney disease: United Kingdom Heart and Renal Protection (HARP)-III-rationale, trial design and baseline data. *Nephrol Dial Transplant*. 2017;32(12):2043-2051
  Chapter 5, 7 and 8
- Haynes R, **Judge PK**, Staplin N, Herrington WG, Storey BC, Bethel A, Bowman L, Brunskill N, Cockwell P, Hill M, Kalra PA, McMurray JJV, Taal M, Wheeler DC, Landray MJ, Baigent C. Effects of Sacubitril/Valsartan Versus Irbesartan in Patients With Chronic Kidney Disease. *Circulation*. 2018;138 (15):1505-1514
  Chapters 7 and 8
- Haynes R, Zhu D, **Judge PK**, Herrington WG, Kalra PA, Baigent C. Chronic kidney disease, heart failure and neprilysin inhibition. *Nephrol Dial Transplant*. 2020; 35(4): 558-564
  Chapters 3, 4 and 8

Other publications containing material covered in this thesis

- Herrington W, Staplin N, **Judge PK**, Mafham M, Emberson J, Haynes R, Wheeler DC, Walker R, Tomson C, Agodoa L, Wiecek A, Lewington S, Reith CA, Landray MJ, Baigent C, SHARP Collaborative Group. Evidence for Reverse Causality in the Association Between Blood Pressure and Cardiovascular Risk in Patients with Chronic Kidney Disease. *Hypertension*. 2017;69(2):314-322
  Chapter 2 and 8

Abstract

BACKGROUND:

The effects of neprilysin inhibition in people with advanced chronic kidney disease (CKD) are unclear. UK Heart and Renal Protection (HARP)-III aimed to examine the effects of sacubitril/valsartan compared with irbesartan, on kidney function, other renal and cardiovascular outcomes and safety in CKD.

METHODS:

UK HARP-III was a randomized trial, including 414 people with CKD with an estimated glomerular filtration rate (GFR) of 20 to 60 mL/min/1.73m^2. Participants were allocated to sacubitril/valsartan or irbesartan. The primary outcome was measured GFR at 12 months. All analyses were intention to treat.

RESULTS:

207 participants were allocated sacubitril/valsartan and 207 irbesartan. At 12 months, there was no difference in measured GFR among those allocated sacubitril/valsartan compared with irbesartan (mean difference -0.1 [SE 0.7] mL/min/1.73m^2). The effect of sacubitril/valsartan did not differ in a range of prespecified subgroups. There was no significant difference in urinary albumin:creatinine ratio (study average difference -9%; 95% CI -18 to 1) or estimated GFR (mean difference 0.1 mL/min/1.73m^2; 95% CI -0.5 to 0.7; P=0.66) over 12 months between treatments. Sacubitril/valsartan, compared with irbesartan, significantly reduced study average systolic and diastolic blood pressure by 5.4 (95% CI 3.4-7.4) and 2.1 (95% CI 1.0-3.3) mmHg respectively. Concentrations of cardiac biomarkers N-terminal of prohormone brain natriuretic peptide and troponin I were by reduced by 18% (95% CI 11-25) and 16% (95% CI 8-23) respectively. Incidence of serious adverse events (29.5% versus 28.5%; RR 1.07; 95% CI 0.75-1.53), non-serious adverse reactions (36.7% versus 28.0%; rate ratio 1.35; 95% CI 0.96-1.90) and renal adverse events were not significantly different between randomized treatments.

CONCLUSIONS:

Over 12 months allocation to sacubitril/valsartan, compared with irbesartan, had no significant effects on kidney function or albuminuria, but did significantly reduce blood pressure and cardiac biomarkers. It was not associated with any major adverse effects in people with advanced CKD.

1 List of abbreviations

AASK	African American Study of Kidney Disease and Hypertension
Aβ	β-amyloid
ACE	Angiotensin-converting enzyme
ACEi	Angiotensin-converting enzyme inhibitor
AD	Alzheimer's dementia
ADHF	Acute decompensated heart failure
AE	Adverse event
AF	Atrial fibrillation
AKI	Acute kidney injury
ALT	Alanine aminotransferase
ANCOVA	Analysis of Covariance
ANP	Atrial natriuretic peptide
APP	Aminopeptidase P
ARNI	Angiotensin receptor-neprilysin inhibition
ARB	Angiotensin receptor blocker
ARSAC	Administration of radioactive substances advisory committee
AST	Aspartate aminotransferase
ATI	Angiotensin I
ATII	Angiotensin II
BMI	Body mass index
BNP	Brain natriuretic peptide
BP	Blood pressure
CAA	Cerebral amyloid angiopathy
CANVAS (-Renal)	Canagliflozin Cardiovascular Assessment Study Program
CCO	Central Coordinating Office
CES	Carboxyl esterases
cGMP	Cyclic guanosine monophosphate
CSG-captopril	Collaborative Study Group-captopril trial
CI	Confidence interval
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease-Epidemiology Collaboration
Cmax	Maximum observed concentration
CNP	C-type natriuretic peptide
CrCl	Creatinine clearance
^{51}Cr-EDTA	^{51}Cromium-ethylenediaminetetraacetic acid
CREDENCE	Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation
CRP	C-reactive protein
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trials of Investigational Medicinal Product
CTSU	Clinical Trials Service Unit & Epidemiological Studies Unit
CV	Cardiovascular
CVD	Cardiovascular disease
DAP	Data analysis plan
DBP	Diastolic blood pressure
DM	Diabetes mellitus
DMC	Data Monitoring Committee
DPPIV	Dipeptidyl peptidase IV
DRI	Direct renin inhibitor
ECE (-1)	Endothelin-converting enzyme (-1)
ECM	Extracellular matrix
eGFR	Estimated glomerular filtration rate

EMA	European Trials Agency
EMPA-REG Outcome	Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients - Removing Excess Glucose (EMPA-REG OUTCOME)
ESKD	End-stage kidney disease
ET (-1)	Endothelin (-1)
EudraCT	European Union Drug Regulating Authorities Clinical Trials
EVALUATE-HF	Effect of Sacubitril-Valsartan vs Enalapril on Aortic Stiffness in Patients with Heart Failure and Reduced Ejection Fraction
FDA	Food and Drug Administration
FGF-23	Fibroblast growth factor 23
Gal-3	Galectin-3
GC	Guanylyl cyclase
GCP	Good Clinical Practice
GDF-15	Growth differentiation factor-15
GFR	Glomerular filtration rate
GMP	Guanosine monophosphate
GTP	Guanosine triphosphate
HbA1C	Haemoglobin A1C
HDL	High density lipoprotein
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HOPE	Heart Outcomes Prevention Evaluation
HR	Hazard ratio
Hs-TnT	Highly sensitive troponin T
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDNT	Irbesartan in Diabetic Nephropathy Trial
IgG	Immunoglobulin G
IL-6	Interleukin 6
IMPRESS	Inhibition of Metallo Protease by Omapatrilat in a Randomized Exercise and Symptoms Study of Heart Failure trial
IOP	Internal operating procedure
IQR	Interquartile range
IRAS	Integrated Research Approval System
ISRCTN	International Standard Randomized Controlled Trial Number
IT	Information technology
ITT	Intention to treat
KCCQ	Kansas City Cardiomyopathy Questionnaire
KDIGO	Kidney Disease: Improving Global Outcomes
KIM-1	Kidney injury molecule
LCC	Local clinical centre
LDL	Low density lipoprotein
LLI	Local lead investigator
LV	Left ventricle/left ventricular
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
MAPK	Mitogen-activated protein kinase
MDRD	Modification of Diet in Renal Disease
MedRA	Medical dictionary for Regulatory Activities
mGFR	Measured glomerular filtration rate
MHRA	Medicines & Healthcare Products Regulatory Agency
MI	Myocardial infarction
MMP-2	Matrix metalloproteinase-2

MMSE	Mini mental state examination
NEP	Neutral endopeptidase or neprilysin
NEPi	Neutral endopeptidase/neprilysin inhibition
NGAL	Neutrophil gelatinase-associated lipocalin
NHS	National Health Service
NICE	National Institute for Clinical Excellence
NO	Nitric oxide
NP	Natriuretic peptide
NPR	Natriuretic peptide receptor
NSAR	Non serious adverse reaction
NT-proANP	N-terminal of the prohormone atrial natriuretic peptide
NT-proBNP	N-terminal of the prohormone brain natriuretic peptide
NYHA	New York Heart Association
OCTAVE	Omapatrilat Cardiovascular Treatment versus Enalapril trial
ONTARGET	Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial
OR	Odds ratio
OVERTURE	Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events trial
PARADIGM-HF	Prospective comparison of ARNi with ACEi to Determine Impact on Global Mortality and morbidity in Heart Failure trial
PARAGON-HF	Prospective Comparison of ARNI with ARB Global Outcomes in HF with Preserved Ejection Fraction
PARAMETER	Prospective Comparison of Angiotensin Receptor Neprilysin Inhibitor with Angiotensin Receptor Blocker Measuring Arterial Stiffness in the Elderly
PARAMOUNT	Prospective comparison of ARNi with ARB on Management Of heart failure with preserved ejection fraction trial
PCR	Protein:creatinine ratio
PIIINP	Collagen III N-terminal propeptide
PIONEER-HF	Comparison of Sacubitril/Valsartan versus Enalapril on Effect on NT-proBNP in Patients Stabilized from an Acute Heart Failure Episode
pmp py	patients per million population per year
PT	Preferred term
R&D	Research and development
RAS	Renin-angiotensin system
RASi	Renin-angiotensin system inhibitor
REC	Research ethics committee
RENAAL	Reduction of Endpoints in NIDDM (non-insulin dependent diabetes mellitus) with the Angiotensin II Antagonist Losartan trial
RR(R)	Risk Ratio or Rate Ratio or Relative Risk (Reduction)
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBP	Systolic blood pressure
SCr	Serum creatinine
SD	Standard deviation
SE	Standard error
SHARP	Study of Heart and Renal Protection trial
SMQ	Standardized MedRA Query
SNP	Single nucleotide polymorphism
SOP	Standard Operating Procedure
SPRINT	Systolic Blood Pressure Intervention Trial
SSAR	Suspected serious adverse reaction

SSH	Salt-sensitive hypertension
sST-2	Soluble form of ST2
T½	Half-life
^{99}mTc-DTPA	^{99}mTechnetium-Diethyl Enetriamine Penta-acetic Acid
TGF-β	Transforming growth factor-β
TNF-α	Tumour necrosis factor
TSC	Trial Steering Committee
uACR	Urine albumin:creatinine ratio
ULN	Upper limit of normal
UK	United Kingdom
UK HARP-III	UK Heart and Renal Protection-III
uPCR	Urine protein:creatinine ratio
VA NEPHRON D	Veterans Affairs Nephropathy in Diabetes
VPI	Vasopeptidase inhibitor
WHO	World Health Organisation

2 Introduction

2.1 Chronic kidney disease

Chronic kidney disease (CKD) is defined by the presence of structural or functional kidney damage present for at least three months.[1,2] CKD may present as a pathological abnormality or with markers of kidney damage.[1] Diagnosis of CKD is based on evidence of one or more of the following: estimated glomerular filtration rate (GFR) less than 60 mL/min/1.73m^2; albuminuria (urine albumin:creatinine ratio [uACR] of 30 mg/g [3mg/mmol] or greater); abnormalities in urinary sediment, histology, or imaging suggestive of kidney disease or damage; electrolyte or other renal tubular disorders; or a history of kidney transplantation.[2,3] CKD is classified into five stages according to estimated GFR (eGFR). Albuminuria is included in classification systems to enable risk stratification of patients in addition to diagnosis of CKD (Table 1).

			Albuminuria category		
			A1	**A2**	**A3**
eGFR† category (mL/min/1.73m^2)			**<3 mg/mmol (<30 mg/g)**	**3-30 mg/mmol (30-300 mg/g)**	**>30 mg/mmol (>300 mg/g)**
G1	Kidney damage with normal or increased eGFR	≥90	51.2	3.4	0.3
G2	Kidney damage with mild decreased eGFR	60–89	36.7	3.0	0.4
G3a	Mild-moderately decreased eGFR	45-59	2.8	0.7	0.2
G3b	Moderate-severely decreased eGFR	30-44	0.6	0.3	0.1
G4	Severely decreased eGFR	15-29	0	0	0.2
G5	Kidney failure	<15 or on dialysis or transplanted	0	0	0.1

Table 1: Prevalence estimates of chronic kidney disease in the United States by stage
†eGFR = estimated glomerular filtration rate
Prevalence estimates are taken from the National Health and Nutrition Examinations Survey III (NHANES III; 1988–1994)[4]

CKD affects between 2% and 17% of the population (depending on the country studied) and rates increase substantially with age.[5,6] Prevalence data from the United States suggest about 5% have moderate CKD (eGFR 30 to 59 ml/min/1.73m^2) and about 0.3% have more advanced CKD (stage 4 or 5; Table 1).[4,6]

CKD is associated with two major hazards: increased risk of progression to end-stage kidney disease (ESKD) requiring treatment with renal replacement therapy (dialysis or transplantation) and premature morbidity and mortality from cardiovascular disease (CVD).[7-9] Progressive decline in renal function is not uncommon and each year the number of prevalent renal replacement therapy patients in the UK increases.[10] There are many aetiologies contributing to ESKD including glomerulonephritis, diabetes, hypertension and polycystic kidney disease with no cause identified in about 16% of cases.[10] As renal function declines, associated complications that may develop include anaemia, bone and mineral disorders, acid-base disturbances, hypertension and dyslipidaemia.[2] CKD has a substantial impact on a patient's quality of life and healthcare expenditure, it is therefore a major public health concern.[11-13]

2.2 Progression of chronic kidney disease

The number of functioning nephrons declines with increasing age. Individuals with reduced nephron mass at birth may reach a lower critical mass of nephrons earlier than those with larger numbers of functioning nephrons, predisposing to CKD.[14,15] Reduced nephron mass impedes functional reserve for adaptation following renal insults and/or injury that may further decrease functioning nephron mass.[14-16]

Following substantial nephron loss, glomerular hyperfiltration and hypertrophy are maladaptive compensatory responses that occur in remaining viable nephrons.[16-18] In animal models of CKD, glomerular hyperfiltration activates the renin-angiotensin system (RAS; Figure 1) and alters glomerular haemodynamics.[19,20] RAS activation raises circulating levels of angiotensin II (ATII) which stimulates aldosterone release, inactivates bradykinin, increases release of noradrenaline and decreases nitric oxide (NO) activity, all of which contribute to potent systemic and renal vasoconstriction.[20-22]

Afferent arteriolar resistance decreases to a greater extent than efferent arteriolar resistance and so, glomerular capillary hydrostatic pressure rises.[16,23] Remaining

nephrons are exposed to increased renal blood flow to maintain an adequate production of filtrate and minimize reductions in glomerular filtration rate.[16,23]

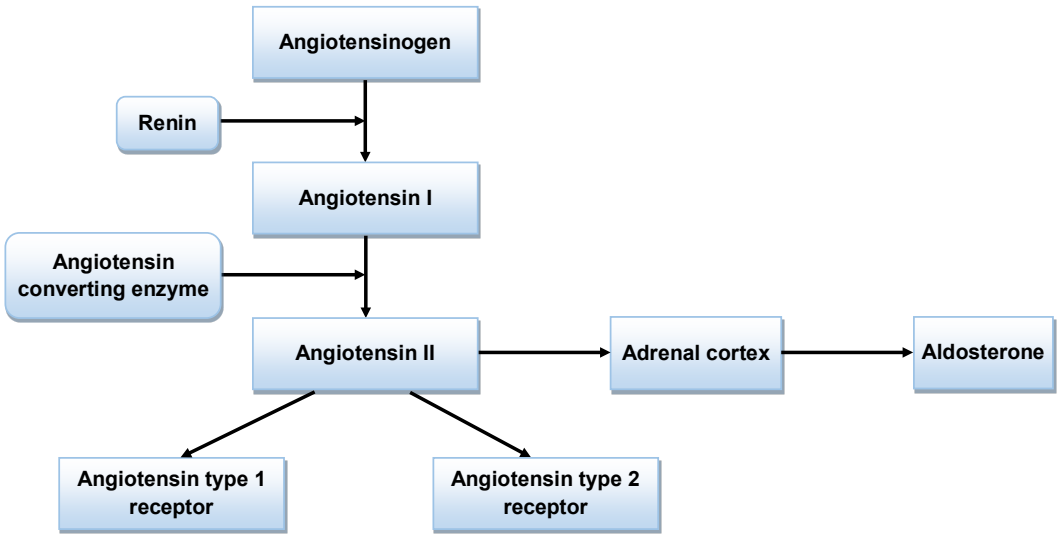


Figure 1: Renin-angiotensin system

Over time these changes and the sustained activation of RAS has detrimental effects that contribute to the development of intra-glomerular and systemic hypertension as well as proteinuria due to increased filtration of plasma proteins.[17,19,23] Systemic hypertension can be a cause, or consequence of CKD and its prevalence increases with falling GFR.[24] In healthy individuals, renal blood pressure is controlled by autoregulation, but if renal auto-regulation is impaired (e.g. due to reduced nephron mass) sustained transmission of raised systemic blood pressure to the kidney increases vascular resistance.[25] Activation of other vasoactive and inflammatory molecules such as endothelin and cyclooxgenase have also been implicated in the development of glomerular hypertension.[17,26]

2.3 Proteinuria and chronic kidney disease

Proteinuria is an important risk factor associated with progression of CKD. Animal studies suggest filtration of proteins (such as albumin) through the glomerulus may be toxic to the kidneys, stimulating the production of inflammatory and vasoactive peptides (including endothelin and ATII), ultimately leading to renal scarring and

dysfunction.[17,27,28] Therefore, the larger the quantity of filtered proteins, the greater the extent and severity of the renal injury that arises, hastening the progression of CKD.[27,28]

Observational studies and meta-analyses have shown both lower eGFR and increasing albuminuria are independently associated with adverse renal outcomes in the general population and in high-risk cohorts.[29-31] An eGFR of less than 60 ml/min/1.73m^2 and rising levels of albuminuria have been associated with increased risk of acute kidney injury, progressive CKD and ESKD.[4,29] However, these findings have been based on results from either animal experiments or observational data from humans.

Increasing albuminuria, independent of eGFR, has been associated with graded increases in risk of ESKD without an apparent threshold. Individuals with an eGFR above 60 mL/min/1.73m^2 and microalbuminuria (uACR greater than 30 mg/g), have substantially increased risk of ESKD, compared to those without albuminuria.[4] Similar risks have been shown in people with diabetes[32] or hypertension,[24] irrespective of their ethnicity.[33]

In a meta-analysis of 693,816 people with CKD (including 557,583 with diabetes), a 30% reduction in albuminuria over 2 years was associated with a 17% (hazard ratio [HR] 0.83; 95% confidence interval [CI] 0.74-0.94) reduction in risk of developing ESKD.[34] An increase in uACR of 43% was associated with a 14% (HR 1.14; 95% CI 1.06-1.22) increase in risk of cardiovascular mortality.[34]

The central role of RAS in the development of intra-glomerular hypertension, systemic blood pressure, proteinuria and progression of CKD led to the development of RAS inhibitors (angiotensin-converting enzyme inhibitors [ACEi] and angiotensin receptor blockers [ARB]; Figure 2).[18]

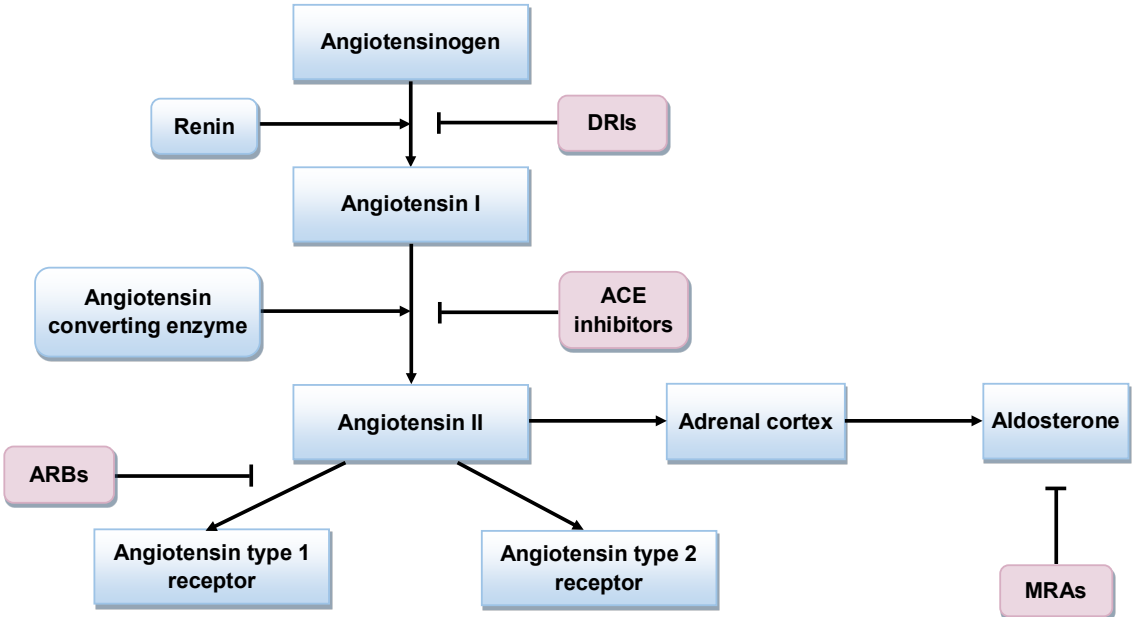


Figure 2: Renin-angiotensin system and sites of therapeutic blockade
ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; DRI = direct renin inhibitor; MRA = mineralocorticoid receptor antagonist

2.4 Current treatments for chronic kidney disease

2.4.1 Renin-angiotensin system blockade

In animal models of renal disease, RAS inhibitors were shown to reduce systemic and intraglomerular pressure, decrease glomerulosclerosis and reduce proteinuria.[35-37] Prevention of glomerular hypertension by RAS blockade was believed to be a key mechanism underlying the renoprotective effect of RAS inhibitors (ACEi's and ARBs).[38]

Several clinical trials have consistently demonstrated that ACEi and ARBs reduce risk of adverse renal outcomes in patients with diabetic and non-diabetic proteinuric CKD (Appendix 1: Summary of trials of targeting blood pressure and albuminuria reductions).[39-42] The Collaborative Study Group (CSG)-Captopril trial randomized people with insulin-dependent diabetes mellitus and proteinuric (500 mg/d or greater) CKD (serum creatinine [SCr] 221 µmol/L or lower) to the ACEi captopril (n=207) or placebo (n=202).[39] Allocation to captopril, compared with placebo, reduced the risk of doubling in SCr (the primary outcome) by 43% (25/207 [12%] versus 43/202 [21%] respectively; risk reduction [RR] 43% [95% CI 6-65]; P=0.007) and risk of dialysis, transplantation or death by 46% (23/207 [11%] versus 42/202 [21%] respectively; RR 46% [95% CI 10-68]; P=0.006).[39] However, despite these reductions 9.7% (20/207) of

participants treated with captopril still progressed to ESKD requiring dialysis or transplantation over a median duration of 1.7 years.[39]

In the Irbesartan in Diabetic Nephropathy Trial (IDNT), irbesartan (an ARB) reduced the risk of the primary composite endpoint of doubling of baseline serum creatinine, ESKD or death by 20% (HR 0.80; 95% CI 0.66-0.97; P 0.03) compared with placebo and, by 23% (HR 0.77; 95% CI 0.63-0.93; P=0.006) compared with amlodipine among people with diabetic nephropathy.[41] In spite of the reduction in risk of ESKD, 14.2% (87/579) of IDNT participants treated with irbesartan still progressed to ESKD during 2.6 years of follow-up.[41] Other trials have shown similar findings.[42]

To address this residual risk, it was hypothesised combining ACE inhibitors and ARBs for maximal RAS inhibition ("dual blockade") could be more effective than either agent alone at reducing albuminuria,[43] risk of progression of CKD to ESKD and cardiovascular events.[44-46] Randomized trials of dual blockade demonstrated greater reductions in albuminuria than either ACEi or ARB alone, but dual blockade did not confer any additional renal or cardiovascular protection.[43-46]

In the Veterans Affairs Nephropathy in Diabetes (VA NEPHRON-D) trial, 1448 people with proteinuric diabetic kidney disease were randomized to receive losartan combined with lisinopril or losartan with placebo.[46] There was no effect on risk of the primary renal outcome (change in eGFR, ESKD, or death) with dual blockade compared with isolated RAS blockade (132/724 [18.2%] versus 152/724 [21%] respectively; HR 0.88 [0.70-1.12]; P=0.30), or on risk of the composite tertiary cardiovascular outcome (risk of myocardial infarction, heart failure or stroke; 134/724 [18.5%] versus 136/724 [18.8%] respectively; HR 0.97 [95% CI 0.76-1.23]; P=0.79).[46]

Furthermore, in VA NEPRON-D dual blockade, compared with isolated RAS blockade, was associated with increased risk of adverse events including hyperkalaemia (72/724 [9.9%] versus 32/724 [4.4%] respectively; HR 2.8 [95% CI 1.8-4.3]; P<0.001) and acute kidney injury ([AKI] 130/724 [18.0%] versus 80/724 [11.0%] respectively; HR 1.7 [95% CI 1.3-2.2]; P<0.001).[46] In the Ongoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial (ONTARGET) comparing ramipril, telmisartan or both, requirement for acute dialysis was 2-fold higher in those randomized to combination therapy compared with ramipril-alone (28/8502 [0.33%] versus 13/8576 [0.15%]; HR 2.19 [95% CI 1.13-4.22]; P=0.020).[44-46] Similar findings were seen when the direct renin inhibitor (DRI; Figure 2), aliskiren, was combined with RAS inhibition as an alternative approach to dual RAS blockade.[47]

2.4.2 Blood pressure lowering

Hypertension is associated with risk of progression to ESKD and cardiovascular events in CKD.[48-51] CKD is both a cause of and, can be caused by hypertension. Two previous randomized trials in CKD populations did not show 'intensive' versus 'standard' blood pressure lowering targets reduced the risk of ESKD but did suggest a benefit in reducing the decline in kidney function in those with baseline proteinuria.[52,53]

The African American Study of Kidney Disease and Hypertension (AASK) trial randomized hypertensive CKD patients (GFR 20 to 65 mL/min/1.73m^2) to either an 'intensive' mean arterial pressure target of 92 mmHg or lower (achieved mean [128/78 mmHg) or, to a 'standard' mean arterial pressure target of 102 to 107 mmHg (achieved mean 141/85 mmHg).[54] From baseline to four years, there was no difference in the mean (SE) decline in GFR between the intensive vs usual treatment arms (2.21 [0.17] mL/min/1.73m^2/year versus 1.95 [0.17] mL/min/1.73m^2/year; P=0.24).[54] The risk of the secondary renal composite outcome of doubling of serum creatinine, ESKD, or death, did not differ between the intensive, compared with standard, blood pressure targets during the trial phase (159/540 versus 169/554 respectively; HR 0.88 [95% CI 0.71-1.09]; P=0.24) or during the longer-term follow-up period ranging between 8.8 to 12.2 years (123/377 versus 116/382 respectively; HR 0.95 [95% CI 0.74-1.23]; P=0.70).[53]

The Modification of Diet in Renal Disease (MDRD) trial randomized 840 patients with CKD (study 1: 585 patients with a GFR of 25-55 mL/min/1.73m^2 and study 2: 255 patients with a GFR of 13-24 mL/min/1.73m^2) to intensive or standard mean arterial blood pressure targets (92 versus 107 mmHg respectively).[55] From baseline to 3 years, there was no difference in the rate of decline in GFR between intensive compared with standard blood pressure lowering in study 1 (10.7 [95% CI 9.1-12.4] mL/min/3 years versus 12.3 [95% CI 10.6-14.0] mL/min/3 years respectively) or in study 2 (3.7 [95% CI 3.1-4.3] mL/min/year versus 4.2 [95% CI 3.6-4.9] mL/min/year respectively).[55] In longer-term follow-up (mean 6.2 years), intensive blood pressure lowering, compared with the standard target, reduced the risk of kidney failure substantially (defined as need for dialysis or kidney transplantation; 268/432 versus 286/408 respectively; HR 0.68 [95% CI 0.57-0.82]; P<0.001).[52]

The Systolic Blood Pressure Intervention Trial (SPRINT) compared an 'intensive' systolic blood pressure target of less than 120 mmHg (achieved blood pressure 121.4/68.7 mmHg) with a 'standard' systolic blood pressure of less than 140 mmHg (achieved blood pressure 136.2/76.3 mmHg) in 9361 adults at increased cardiovascular risk.[56] Mean (SD) eGFR amongst the overall cohort was 71.8 (20.7)

mL/min/1.73 m^2 and in those with CKD (eGFR less than 60 mL/min/1.73m^2) at baseline, mean (SD) eGFR was 47.9 (9.5) mL/min/1.73m^2 and mean (SD) uACR was 42.6 (165.8) mg/g.[56]

Amongst 2646 people with CKD at baseline (eGFR 20 to 60 mL/min/1.73m^2) there was no difference in the occurrence of the secondary composite renal outcome (reduction in eGFR of 50% or more, long term dialysis, or kidney transplantation) between the intensive, compared with standard, blood pressure lowering targets (14/1330 [1.1%] versus 15/1316 [1.1%] respectively; HR 0.89 [95% CI 0.42-1.87]; P=0.76).[56] There was no effect on development of albuminuria amongst those with CKD (49/526 [9.3%] intensive versus 59/500 [11.8%] standard; HR 0.72 [95% CI 0.48-1.07]; P=0.11) or in those without CKD (110/1769 [6.2%] intensive versus 135/1831 [7.4%] standard; HR 0.81; [95% CI 0.63-1.04]; P=0.10) during the trial.[56]

Intensive, compared with standard, blood pressure lowering was associated with a substantially increased risk of acute kidney injury (193/4678 [4.1%] versus 117/4683 [2.5%] respectively; HR 1.66; P<0.001), hypotension (110/4678 [2.4%] versus 66/4683 [1.4%] respectively; HR 1.67; P=0.001) and electrolyte abnormalities (144/4678 [3.1%] versus 107/4683 [2.3%] respectively; HR 1.35; P=0.02).[56]

A meta-analysis of 11 trials including 9287 participants with CKD, suggested intensive blood pressure lowering reduced the risk of a composite renal failure outcome (50% decline in GFR and doubling of the serum creatinine or ESKD) by 17% (HR 0.82; 95% CI 0.68-0.98), and reduced the risk of ESKD-alone by 18% (HR 0.79; 95% CI 0.67-0.93).[57] Subgroup analysis showed substantial heterogeneity (P for heterogeneity = 0.006) in the effect of intensive blood pressure lowering by baseline proteinuria, with a 27% (HR 0.73; 95% CI 0.62-0.86) reduction in the composite renal failure outcome in those with proteinuria but no apparent effect in those without proteinuria (HR 1.12; 95% CI 0.67-1.87).[57] A meta-analysis including 613,815 participants, showed each 10 mmHg reduction in systolic blood pressure was associated with a non-significant 5% (RR 0.95; 95% CI 0.84-1.07) reduction in risk of ESKD.[58]

Strict control of blood pressure and proteinuria is desirable in patients with CKD given the results from the MDRD and AASK trials. The Kidney Disease Improving Global Outcomes (KDIGO) guidelines previously recommended a blood pressure target of less than 130/80 in those with proteinuria or diabetes mellitus (DM).[59] In light of more recent randomized evidence demonstrating benefits of lower blood pressure targets, the new guidance on blood pressure management in people with CKD is likely to recommend a systolic blood pressure target of less than 120 mmHg.[60] However, the targets will need

to be individualised in people who may be at increased risk of harm from more aggressive blood pressure lowering, such as the elderly.[60]

2.4.3 Sodium-glucose co-transporter 2 inhibition and effects on renal outcomes

Sodium-glucose co-transporter 2 (SGLT2) inhibition has emerged as a new potential therapy for prevention of both cardiovascular and renal outcomes in people with diabetic kidney disease. SGLT2 is a sodium dependent glucose transporter protein located in the first segment of the proximal renal tubule and is responsible for 80-90% of filtered glucose reabsorption.[61]

SGLT2 inhibition has been shown to reverse the maladaptive changes that occur in the kidney with poorly controlled diabetes resulting in reduced glucose and sodium reabsorption, thereby increasing distal sodium delivery to the macula densa in the kidney.[61,62] Increased urinary glucose excretion and natriuresis occurs and local RAS activation is suppressed, resulting in activation of tubuloglomerular feedback. Tubuloglomerular feedback vasoconstricts the afferent arteriole which reduces renal perfusion pressure, intraglomerular hypertension and glomerular filtration rate.[61,62] The combined effect of all these changes is a reduction in systemic (as well as intraglomerular) blood pressure, diuresis, weight loss and reduced circulating glucose concentrations.[62]

The Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients - Removing Excess Glucose (EMPA-REG OUTCOME) trial, was the first large-scale randomised trial to demonstrate a substantial benefit with SGLT2 inhibition on renal and cardiovascular outcomes.[63] People with type 2 diabetes mellitus and eGFR above 30 mL/min/1.73m^2 were randomized to empagliflozin (SGLT2 inhibitor) or placebo. Among those with CKD at baseline, mean±SD eGFR was 67.1±7.9 mL/min/1.73m^2 and 40% of the overall cohort had albuminuria.[63,64]

Allocation to empagliflozin, compared with placebo, reduced the risk of incident or worsening nephropathy by a substantial 39% (525/4124 [12.7%] versus 388/2061 [18.8%] respectively; HR 0.61 [95% CI 0.53-0.70]; P<0.001).[64] Empagliflozin, compared with placebo, had a similar effect in people with prevalent CKD (eGFR less than 60 mL/min/1.73m^2) and/or macroalbuminuria (207/998 [20.7%] versus 161/507 [31.8%]; HR 0.58 [95% CI 0.47-0.71]; P<0.001).[64] Empagliflozin, compared with placebo reduced the risk of the post hoc composite renal outcome (doubling of SCr, initiation of RRT, or death from renal disease) by 46% (81/4645 [1.7%] versus 71/2323

[3.1%]; HR 0.54 [95% CI 0.40-0.75]; P<0.001), with no heterogeneity in the treatment effect by baseline albuminuria (P for interaction = 0.51) or eGFR (P for interaction = 0.18).[64]

Empagliflozin produced an acute decline in eGFR in the first month post-randomization (mean±SE adjusted eGFR fall 0.82±0.04 mL/min/1.73m^2 with 25 mg empagliflozin versus 0.01±0.04 mL/min/1.73m^2 with placebo).[64] Thereafter, the annual rate of decline was slower with empagliflozin compared with placebo (0.19±0.11 versus 1.67±0.13 mL/min/1.73m^2 respectively; P<0.001).[64] At one month following cessation of randomized treatment, empagliflozin, compared with placebo, was associated with greater adjusted weekly increases in eGFR (0.55±0.04 mL/min/1.73m^2 [with 25 mg empagliflozin] versus 0.04±0.04 mL/min/1.73m^2 respectively; P<0.001).[64]

Since the publication of the EMPA-REG trial results, two additional trials of SGLT2 inhibition in people with diabetic nephropathy have followed, examining a renal primary outcome. The Canagliflozin Cardiovascular Assessment Study (CANVAS) Program consisted of two randomized trials (CANVAS and CANVAS-Renal) comparing canagliflozin (n=5795) with placebo (n=4347) in people with type 2 diabetes at high risk of CV events.[65-67] Canagliflozin, compared with placebo, reduced the risk of the pre-specified renal composite outcome (sustained doubling of SCr, ESKD or death from renal disease) in all participants with type 2 diabetes mellitus enrolled in the CANVAS Program by 47% (1.5 versus 2.8 per 1000 patient-years; HR 0.53; 95% CI 0.33-0.84) with no difference in the treatment effect across a range of subgroups.[66] Allocation to canagliflozin, compared with placebo, was associated with a slower decline in eGFR (between group difference 1.2 mL/min/1.73m²/year; 95% CI 1.0-1.4) and reduced albuminuria by 18% (between group difference 18%; 95% CI 16-20).[66]

The Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial randomized 4401 people with type 2 diabetes and proteinuric CKD (uACR 300 to 5000 mg/g and eGFR 30-90 mL/min/1.73m^2), to canagliflozin (n=2202) or placebo (n=2199).[67] Allocation to canagliflozin, compared with placebo, reduced the risk of the primary composite outcome (ESKD [dialysis, transplantation, or eGFR of less than 15 mL/min/1.73m^2], doubling of SCr or, either a cardiovascular or renal death) by 30% (245/2202 [11.1%] versus 340/2199 [15.5%] respectively; HR 0.70 [95% CI 0.59-0.82]; P=0.00001) with no heterogeneity in the treatment effect in a rage of pre-specified subgroups.[67] Allocation to canagliflozin, compared with placebo, was associated with a slower decline in eGFR (between group

difference 1.52 mL/min/1.73m^2/year; 95% CI 1.11-1.93) and a 31% (95% CI 26-35) reduction in albuminuria.[67]

Although results from trials of SGLT2 inhibition have shown significant reductions in renal outcomes, these benefits have only been shown in people with diabetic nephropathy. For people with non-diabetic CKD, RAS inhibition currently remains the cornerstone of treatment to reduce risk of progression to ESKD. Despite treatment with RAS inhibition, a substantial residual risk of progression to ESKD remains. Trials of SGLT2 inhibition in people with non-diabetic CKD, including The Study of Heart and Kidney Protection With Empagliflozin (EMPA-KIDNEY), are currently underway to examine whether these drugs have similar effects on renal outcomes to people with diabetic nephropathy but, at present there remains an unmet need for more effective strategies to address this residual risk.[68,69]

2.5 Cardiovascular disease in chronic kidney disease

Whereas only a minority of patients with CKD progress to ESKD, cardiovascular disease (CVD) is much more common with many patients dying from cardiovascular events before the onset of ESKD.[8,70-72] In the UK, CVD accounts for about a quarter of all deaths amongst those receiving renal replacement therapy and the life expectancy for patients with CKD is substantially reduced compared to those of a similar age in the general population, even among young patients.[8,10]

Risk of CVD in CKD is associated with both “traditional” (including hypertension, diabetes, obesity, smoking and dyslipidaemia) and “non-traditional” risk factors which are related to manifestations of progressive CKD.[60,73-78] Non-traditional risk factors include abnormalities in mineral bone disease (including abnormal calcium and phosphate balance, hyperparathyroidism, vitamin D deficiency, increases in fibroblast growth factor-23 [FGF-23]), inflammation (such as C-reactive protein), anaemia, albuminuria and uraemia.[4,79-83]

A variety of pathological changes contribute to the excess cardiovascular risk in CKD including: atherosclerosis; arteriosclerosis (resulting in non-compliant vasculature, increased pulse pressure, left ventricular hypertrophy [LVH] and decreased coronary perfusion); hypertension; RAS and sympathetic nervous system hyperactivity;

structural heart disease (including LVH and left ventricular dilatation) and; development of heart failure (HF).[8,71,72,84,85]

As CKD progresses, the manifestation of CVD changes from atherosclerotic disease (i.e. myocardial infarction [MI], ischaemic stroke) to non-atherosclerotic disease (characterised by arteriosclerosis and structural heart disease).[86-88] Among patients with advanced CKD (stages 4-5) up to 50% have echocardiographic evidence of abnormal cardiac structure which is often clinically asymptomatic.[89,90] Non-atherosclerotic disease manifests clinically as heart failure and has a high incidence of sudden cardiac death.[8,88]

Observational studies have previously reported 'U'- or 'J'-shaped associations between blood pressure and cardiovascular risk in people with advanced CKD with an increased CVD risk at low to normal blood pressure.[24,50,51] This contrasts with the positive log-linear associations with ischemic heart disease, stroke, and heart failure mortality observed amongst apparently healthy adults.[91]

The Study of Heart and Renal Protection (SHARP) trial, randomized 9270 patients with CKD, without a prior history of myocardial infarction or coronary revascularization, to simvastatin and ezetimibe or placebo.[75] In post-hoc analyses, amongst the 7278 participants who reported no previous history of CVD, there was a positive log-linear association between increasing systolic blood pressure and risk of CVD risk.[92] Each 10 mmHg increase in usual systolic blood pressure was associated with a 16% (HR 1.16; 95% CI 1.08-1.25) increase in cardiovascular risk.[92]

In the subgroup of patients with an additional troponin I (a cardiac biomarker) measurement, in the individuals with a troponin I concentration of 0.01 ng/mL or lower, each 10 mmHg increase in systolic blood pressure was associated with a 27% (HR 1.27; 95% CI 1.11-1.44) increase in cardiovascular risk.[92] The effect of systolic blood pressure on risk of atherosclerotic and non-atherosclerotic cardiovascular events was similar as was the effect in those on dialysis and non-dialysis participants.[92] The association between diastolic blood pressure and risk of CV events was U-shaped irrespective of prior history of CVD or, troponin I result in those without prior CVD.[92]

The presence of a positive log-linear association between systolic blood pressure and cardiovascular events in patients with advanced CKD at the lowest risk of CVD, suggests reverse causality is a possible explanation for the previously observed U or J-shaped associations in people with advanced CKD.[24,50,51] Long-standing hypertension causes changes in cardiac structure and function that lowers blood

pressure and simultaneously increases CV risk.[93] The results suggest that blood pressure may be a causal risk factor for both atherosclerotic and non-atherosclerotic CVD in people with CKD, as in other populations.[92] Large-scale randomized trials in people with CKD are therefore required to assess the effects of intensive blood pressure lowering on CVD and renal outcomes to identify new strategies of reducing the significant burden of CVD in CKD.

Observational studies have also shown a log-linear association between albuminuria and all-cause or cardiovascular mortality without an apparent threshold effect (on a log-log scale), independent of eGFR and other cardiovascular risk factors.[81,94] In people with declining eGFR (below 75 ml/min/1.73m^2), even normoalbuminuria (uACR 10 to 29 mg/g) has been shown to be associated with increased cardiovascular risk compared with an eGFR greater than 90 mL/min/1.73m^2 and/or uACR less than 10 mg/g.[29,81,94] In individuals with an eGFR of 30-44 mL/min/1.73m^2 and macroalbuminuria, the risk of cardiovascular mortality increased 6-fold in general population (HR 6.10; 95% CI 4.08-9.10) and high-risk cohorts (HR 6.00; 95% CI 4.40-8.18).[81,94] Other studies have shown similar associations, highlighting the significant burden of CVD in CKD.[24,32,33]

In a meta-analysis of over 267,000 people with a history of hypertension, diabetes, or cardiovascular disease (and therefore at an increased risk of CKD), compared with an eGFR of 60 mL/min/1.73m^2 or above, people with an eGFR of 45 or 15 mL/min/1.73m^2 had increased risk of cardiovascular mortality (eGFR 45 mL/min/1.73m^2 HR 1.73 [95% CI 1.49-2.00] and eGFR 15 mL/min/1.73m^2 HR 3.08 [95% CI 1.89-5.01]).[94] In people with albuminuria the risk of cardiovascular mortality increased linearly with each log increase in albuminuria: compared with albuminuria of 5 mg/g, HR for microalbuminuria (30 mg/g) was 1.55 (95% CI 1.30-1.86]) and macroalbuminuria (300 mg/g) 2.59 (95% CI 1.95-3.44).[94]

2.6 Prevention of cardiovascular complications

The excess risk of CVD outcomes and associated mortality in people with CKD highlights the need for interventions and therapies that can address this risk. Several interventions have been trialled including lipid lowering, RAS blockade, intensive blood pressure lowering and SGLT2 inhibition.

2.6.1 Lipid lowering

The SHARP trial demonstrated lowering low-density lipoprotein (LDL) cholesterol with simvastatin plus ezetimibe, compared with placebo, produced a 17% (526/4650 [11.3%] versus 619/4620 [13.4%] respectively; rate ratio [RR] 0.83 [95% CI 0.74-0.94]; P=0.0021) proportional reduction in major atherosclerotic events (defined as non-fatal myocardial infarction, or cardiac death, stroke, or arterial revascularisation) in patients with CKD including those on dialysis and in those with renal transplants.[75]

2.6.2 Renin-angiotensin system blockade

In the Reduction of Endpoints in Non-Insulin Dependent Diabetes Mellitus with the Angiotensin II Antagonist Losartan (RENAAL) trial, there was no difference in CV events with losartan compared with placebo (247/751 [32.9%] vs 268/752 [35.2%]; P=0.26).[42] Similarly, in the IDNT trial, there was no significant reduction in risk of the composite cardiovascular endpoint with irbesartan compared with either placebo (RR 0.91; 95% CI 0.72-1.14; P=0.40) or with amlodipine (RR 1.03; 95% CI 0.81-1.32; P=0.78).[41]

In the general population, RAS inhibitors reduce the risk of cardiovascular events and, meta-analyses have suggested that the mechanism extends beyond reductions in blood pressure.[95,96] However, randomized trials such as RENAAL and IDNT in patients with CKD have not shown RAS inhibition has the same benefits on CV outcomes and this may be because they have not been large enough to show such effects.[97]

2.6.3 Intensive blood pressure lowering

Intensive blood pressure lowering on CV outcomes has been trialled in the general population and in people with CKD.[76] Previous trials in CKD populations did not show significant reductions in CV events with intensive blood pressure lowering, but this may be because they were not adequately powered to do so (Appendix 2: Table of intensive versus standard blood pressure lowering).[53,55,98]

The SPRINT trial randomized 9361 to either intensive (n=4678) or standard (n=4683) blood pressure lowering (systolic blood pressure less than 120 mmHg versus less than 140 mmHg respectively).[56] The primary outcome was a composite of myocardial infarction, acute coronary syndrome, stroke, heart failure, or CV mortality.[56] The trial was stopped early after median 3.26 years follow-up as allocation to intensive, compared with standard, blood pressure lowering reduced the rate of the primary

composite outcome by a highly significant 25% (243/4678 [5.2%] versus 319/4683 [6.8%]; HR 0.75 [95% CI 0.64-0.89]; P<0.001).[56] Allocation to intensive, compared with standard, blood pressure lowering reduced all-cause mortality by 27% (155/4678 [3.3%] versus 210/4683 [4.5%]; HR 0.73; [95% CI 0.60-0.90]; P=0.003).[56]

Subgroup analyses from SPRINT assessing the treatment effect of a lower vs higher systolic blood pressure target in people with CKD (108/1330 [8.1%] versus 126/1316 [9.6%] respectively; HR 0.82 [95% CI 0.63-1.07]) compared to those without CKD (135/3348 [4.0%] versus 193/3367 [5.7%]; HR 0.70 [95% CI 0.56-0.87]) found no significant heterogeneity in risk of the primary outcome between the two groups (P for interaction = 0.32).[56] However, the treatment effect was smaller in those with CKD as it was not powered to detect an effect in specific subgroups. The SPRINT results suggest intensive blood pressure lowering could be of significant benefit in reducing CVD outcomes in people with CKD and highlight the need for undertaking adequately powered randomized trials of this intervention in people with CKD.

2.6.4 Sodium-glucose co-transporter 2 inhibition

The EMPA-REG trial primary outcome was a composite of cardiovascular mortality, nonfatal MI, or nonfatal stroke.[63] Allocation to empagliflozin, compared with placebo, reduced the risk of the primary outcome by 14% (490/4687 [10.5%] versus 282/2333 [12.1%] respectively; HR 0.86 [95% CI 0.74-0.99]; P for non-inferiority <0.001 and P for superiority = 0.04).[63] Empagliflozin, compared with placebo, reduced rates of hospitalization for heart failure by 35% (126/4687 [2.7%] versus 95/2333 [4.1%] respectively; HR 0.65 [95% CI 0.50-0.85]; P = 0.002) and all-cause mortality by 32% (269/4687 [5.7%] versus 194/2333 [8.3%] respectively; HR 0.68 [95% CI 0.57-0.82]; P<0.001).[63]

In CREDENCE, cardiovascular events were studied as secondary outcomes.[67] Systolic and diastolic blood pressure were lower in those allocated canagliflozin compared with placebo (3.30 [95% CI 2.73-3.87] and 0.95 [95% CI 0.61-1.28] mmHg respectively).[67] Canagliflozin, compared with placebo, reduced the risk of cardiovascular mortality or hospitalization for heart failure by 31% (179/2202 [8.1%] versus 253/2199 [11.5%] respectively; HR 0.69 [95% CI 0.57-0.83]; P<0.001) and, risk of CV mortality, myocardial infarction, or stroke by 20% (217/2202 [9.9%] versus 269/2199 [12.2%] respectively; HR 0.80 [95% CI 0.67-0.95]; P=0.01).[67]

Currently, no treatments have been shown to be beneficial in reducing non-atherosclerotic CV complications (such as heart failure) associated with CKD in people

without diabetes and so this remains a significant area of unmet clinical need.[8] There is therefore substantial need for new therapeutic strategies that could improve both the risk of progression to ESKD and development of CVD.

One therapeutic strategy has been to target the natriuretic peptide (NP) system. The NP system is a compensatory neurohormonal pathway that counter-regulates RAS and the sympathetic nervous system. Therefore, enhancing the activity of NPs, by raising circulating levels of NPs, would be a beneficial strategy in disease states in which there is excess RAS activation and relative NP deficiency such as CKD, hypertension and CVD.

3 Natriuretic peptide system and neprilysin

3.1 Natriuretic peptides

Natriuretic peptides (NPs) are a family of peptides with short half-lives and similar chemical structures.[99] In humans, three key NPs exist: atrial (ANP), brain (BNP) and C-type (CNP).[99,100] ANP and BNP are predominantly synthesised and released from atrial and ventricular myocytes respectively in response to cardiac atrial distension from raised venous pressure.[99,101] CNP is predominantly expressed in endothelial cells in response to cytokine and endothelium-dependent agonists and has a major role in chondrocyte differentiation and bone formation and also has vasodilatory and anti-fibrotic effects.[102,103]

All three NPs are formed as pre-pro-peptides and the signal peptide is cleaved to form pro-peptides. ANP pro-peptide undergoes a further proteolytic cleavage to convert the 126-amino acid[101,104,105] precursor into two smaller peptide fragments,[99,102] a 98-amino acid N-terminal-fragment (NT-proANP$_{1-98}$) and the biologically active 28-amino acid carboxy-terminal fragment (ANP$_{99-126}$).[99,104,105] Pro-ANP is cleaved by the enzyme corin which is expressed in renal and cardiac tissue.[106,107] Variations in a minor allele of the corin gene (the corin I555 [P568] allele) may be associated with higher levels of blood pressure, hypertension and cardiac hypertrophy in African American individuals.[107,108]

In the kidney, ANP precursor expression produces a NP called urodilatin (a 32-amino acid NP [ANP$_{95-126}$]) from distal renal tubular cells, which regulates sodium and water excretion.[99,101,104,109,110] Cardiovascular effects of ANP include inhibition of endothelin production, proliferation of smooth muscle cells and myocardial hypertrophy.

BNP is produced as the pro-hormone pro-BNP$_{1-108}$. Cleavage by the enzymes corin and furin produces the active molecule BNP$_{1-32}$ and the inactive N-Terminal of prohormone BNP$_{1-76}$ (NT-proBNP).[106,111] Circulating levels of NT-proBNP are elevated in heart failure and levels of this peptide are used as a biomarker to assess disease activity. Specific actions of BNP include: coronary vasodilatation, myocardial relaxation, proliferation of cardiac myocytes and inhibition of cardiac fibroblasts, all of which act to prevent cardiac remodelling, diastolic dysfunction and heart failure.[112] Both ANP and BNP also downregulate genes encoding substances regulating fibrosis (e.g. collagen, fibronectin) and inflammation (e.g. interleukin [IL]-6, tumour necrosis factor [TNF]-α).[112]

NPs exert their physiological effects via natriuretic peptide receptors (NPRs). Three receptors have been identified: NPR-A, -B and -C. Both ANP and BNP predominantly act via NPR-A (also known as guanylyl cyclase [GC]-A) and CNP has highest affinity for NPR-B (GC-B).[99,102] NPs exert their actions through binding to NPR-A and NPR-B which activates cyclic guanosine monophosphate (cGMP)-dependent signalling (Figure 3).[104,113] NPR-C is a clearance receptor for NPs that is not coupled to guanylyl cyclase.

NPs have a range of beneficial actions (Figure 3)[81,92-94] and circulating levels of NPs are upregulated in disease states such as CKD and heart failure to counteract pathways such as RAS and the sympathetic nervous system.

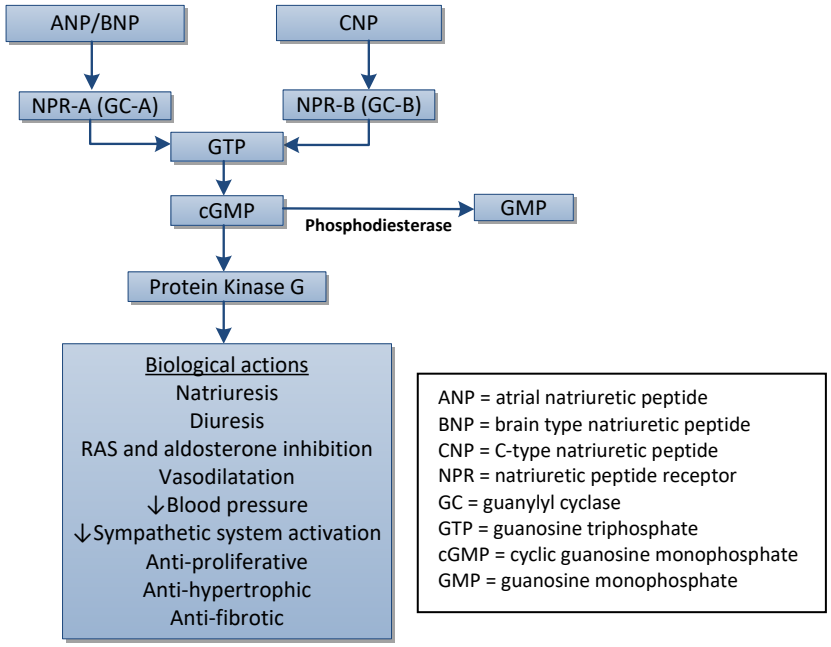


Figure 3: Mechanism of action of natriuretic peptides and neprilysin

3.1.1 Actions of natriuretic peptides in the kidney

NPs have several renal (through their actions on glomeruli and tubules) and cardiovascular effects that contribute to salt and water homeostasis and blood pressure regulation (Figure 3).[105,114,115] Urodilatin has direct effects on the distal tubule and collecting ducts (acting via a paracrine mechanism) contributing to natriuresis and diuresis.[104,109,110,116] Urodilatin inhibits sodium reabsorption to a greater extent than ANP possibly through higher affinity binding to the NPR-A receptor.[116]

ANP increases renal perfusion through pre-glomerular afferent arteriolar vasodilatation and post-glomerular efferent arteriolar vasoconstriction which results in increased intraglomerular capillary pressure, filtration fraction and GFR.[117] Binding of ANP to receptors on glomerular mesangial cells, activates guanylyl cyclase receptors which counteract ATII-induced contraction of mesangial cells, thereby increasing the capillary surface area for filtration and producing a marked diuresis.[118,119]

ANP also inhibits the production and actions of angiotensin-II, renin release (which reduces RAS activation) and the actions of aldosterone and anti-diuretic hormone (ADH) at the collecting duct (Figure 1).[101,104,105,120,121] GFR is maintained, despite increased distal sodium delivery, by decreased proximal sodium reabsorption and increased sodium excretion, giving rise to increased urinary flow rates.[120]

Animal models lacking the pro-ANP gene (resulting in an inability to produce ANP) develop salt-sensitive hypertension (SSH) compared to the wild-type.[122,123] Heterozygotes with reduced circulating ANP levels can also develop SSH although changes in plasma renin activity and dietary salt intake may also contribute.[123] Gene delivery of ANP (using an adenovirus with the human ANP gene attached [Ad.RSV-cANP]) to mice with SSH resulted in reduced blood pressure, renal injury, cardiac hypertrophy and stroke rates.[124,125]

Two single nucleotide polymorphisms (SNP), rs5068 and rs1938358, in the ANP and BNP genes (NP precursor A [NPPA] and B [NPPB] genes respectively) are associated with increased circulating levels of proANP and proBNP respectively, lower blood pressure and risk of hypertension.[126] The rs5068 SNP may also be associated with an improved metabolic profile.[127]

These findings suggest that augmenting circulating levels of NPs could lead to improved clinical outcomes in CKD and CVD.

3.1.2 Raising levels of circulating natriuretic peptides

Studies of patients with advanced CKD and those receiving haemodialysis demonstrated raised ANP levels in these patients compared with healthy controls. Release of ANP is believed to be triggered by volume expansion or fluid overload.[128-130]

In dialysis patients, levels of ANP are much higher prior to haemodialysis and subsequently fall with ultrafiltration (with net fluid removal).[129,130] Therefore, NPs may

have a substantial physiological role in the regulation of fluid balance and blood pressure in people with CKD.

In patients with advanced CKD, infusions of ANP were trialled as a method of raising circulating levels of NPs with conflicting results. Some studies reported no significant increases in GFR and renal plasma flow, whilst others reported the opposite effects with no effect on filtration fraction.[128] There may be several reasons for the inconsistencies including the dose of ANP used in each study the small sample sizes in these non-randomized studies and the extremely short follow-up periods.[131]

Reductions in blood pressure and plasma renin levels (as a marker of RAS activity) were seen despite reduced nephron mass.[128,132] In a study of eight patients with nephrotic syndrome (proteinuria between 4 to 9 g/24 hours), low-dose and high-dose ANP infusions were compared.[133] Urinary albumin and sodium excretion increased significantly in both groups, with a greater degree of albuminuria developing in those given higher doses of ANP.[133] Immunoglobulin G (IgG) excretion increased proportionally to albumin excretion.[133] Similar increases in albuminuria were found in studies, where ANP was administered to patients with insulin-dependent diabetes and microalbuminuria.[132,134]

The increases in albumin excretion may be mediated through enhanced permeability of the glomerular filtration barrier, changes in glomerular haemodynamics (resulting in increased glomerular capillary hydraulic pressure) and additional relaxation of mesangial cells causing an increase in the glomerular filtration surface area.[134,135] The permeability of the filtration slit membranes attached to podocytes (which possess ANP receptors) may also be increased by ANP, allowing albumin and other high molecular weight molecules to be filtered more freely.[133-135]

Infusions of recombinant ANP (carperitide) and BNP (nesiritide) have been tested in patients with cardiac disease. However, their clinical utility in raising NP levels has been limited by short bioavailability, a need for parenteral administration, profound symptomatic hypotension and a lack of clinical benefit in randomized trials.[136,137]

The difficulties with infusions of NPs led to the development of drugs that raise levels of NPs by inhibiting the key enzyme responsible for their degradation, neprilysin (also known as neutral endopeptidase).[99]

3.2 Neprilysin

Neprilysin (NEP) is a membrane-bound zinc-containing metalloproteinase.[99,100] It is a 90 kDa glycoprotein with widespread tissue distribution (including the brain, vascular endothelium, smooth muscle, cardiac myocytes and neutrophils) but has greatest abundance in the brush border of proximal renal tubular cells.[100,103,121,138] It is believed that prevention of breakdown of ANP directly at the brush-border within the kidney enhances natriuretic activity by inhibition of sodium reabsorption in the medullary collecting duct.

In addition to NPs, NEP is responsible for processing and degrading a range of other vasoactive peptides including ATII, bradykinin, endothelin-1, substance P, adrenomedullin and amyloid.[121,138-140]

NEP plays an important role in the formation and breakdown of the vasoconstrictor endothelin (ET), through conversion of big-ET into endothelin-1 (ET-1), and regulating vascular tone.[138,140-142] The final step in processing of ET-1 is catalysed by endothelin-converting enzyme (ECE) which shares sequence homology with NEP. NEP inhibition (NEPi) attenuates the activity of ECE-1 which further enhances the activity of ANP.[138] The net action of NEP depends on the balance between vasoconstrictor and vasodilatory peptides.

The wide range of potentially therapeutic actions of NPs and the limitations with some methods of raising levels of NPs, led to the development of agents that inhibited NEP.

3.3 Neprilysin inhibition

Short-term studies of orally-active NEP inhibitors (NEPi) in healthy human volunteers, demonstrated NEPi resulted in increased urinary and plasma cGMP, plasma ANP (suggesting adequate NEPi was achieved), natriuresis and vasodilation with minimal RAS activation.[143,144] Candoxatrilat was one of the first NEP inhibitors to be produced and tested in a range of patients with CKD,[145] essential hypertension[146,147] and heart failure.[148]

Candoxatrilat was compared with placebo in 24 patients with normal (mean±SD GFR 103±8 mL/min), moderately (GFR 64±6 mL/min) and severely (16±2 mL/min) impaired

renal function (excluding patients on dialysis) in a cross-over study.[145] Compared with placebo, candoxatrilat produced substantial elevations in plasma ANP and urinary cGMP and, a pronounced natriuresis occurred in all three groups.[145]

In contrast to the renal effects noted with NP infusions, no changes in GFR, renal plasma flow or blood pressure were seen.[145] A marked increase in albuminuria emerged in those with severely impaired renal function and may have been due to the effects of ANP on renal vasculature, increasing filtration fraction and transglomerular albumin transport.[128,132,133,145]

Such increases in albuminuria with NEPi, particularly in patients with CKD, would be of great concern if they were shown to persist in longer-term studies of NEPi since albuminuria has been shown to be associated with increased risk of progression of CKD.[29-31] However, all of the studies of NEPi included only very small numbers of participants that were treated and followed-up and for very short timespans to be able to provide reliable information on the effects of NPs in patients with advanced CKD and albuminuria.

In longer term studies, chronic NEPi did not translate into clinically meaningful reductions in blood pressure. Since NEPi impairs the degradation of ATII, any blood pressure and natriuretic effects were attenuated by compensatory up-regulation of the RAS and sympathetic nervous system activity (as well as changes in sodium and endothelin).[140,147,149]

The beneficial renal and cardiovascular effects of NEPi were shown to be enhanced when combined with simultaneous RAS inhibition.[104] This led to the development of dual NEP/RAS inhibitors to counteract the physiological responses that dampen the effects of isolated NEP inhibition.[150]

3.4 Combined neprilysin and renin-angiotensin converting enzyme inhibitors (vasopeptidase inhibitors)

Dual inhibition of NEP and RAS as a single combined treatment was a significant step forward in the development of potential treatments for preventing progression of renal and cardiovascular disease. Initial combinations consisted of a NEPi with an ACEi (working synergistically) and this new class of drugs were termed vasopeptidase

inhibitors (VPIs).[151] Many different compounds were produced and trialled in humans (Table 2) with omapatrilat the most widely studied drug.[139,152,153]

Vasopeptidase inhibitor	Population studied	Year
Phase II studies		
MDL-100240	Healthy volunteers	2000
Z13752A	Healthy volunteers	2000
Gemopatrilat	Healthy volunteers	2001-2004
Phase III studies		
Sampatrilat	Hypertension	1998-99
Omapatrilat	Hypertension, heart failure and cardiovascular disease	1994-2004

Table 2: Examples of vasopeptidase inhibitors produced and studied in humans

In studies of healthy volunteers, omapatrilat was well tolerated and significant increases in urinary excretion of ANP and cGMP were seen compared with ACEi or placebo.[139,152,153] Omapatrilat produced potent ACE inhibition (decreasing levels of angiotensin-II) and reductions in systemic blood pressure.[153] Renal effects included marked renal vasodilatation (with increased renal blood flow) without associated changes in GFR, and decreases in filtration fraction.[153]

Evidence for a potential role of VPIs in CKD came primarily from studies of NEPi in animal models of renal disease and the results of renal outcomes in trials of NEPi in heart failure and hypertension. No clinical outcome trials of VPIs were performed in people with CKD.

In a variety of animal models of hypertension including SSH, stroke-prone spontaneously hypertensive rats and renovascular hypertension, combined NEP/RAS inhibition resulted in greater reductions in blood pressure and vascular remodelling compared with isolated RAS inhibition or control.[141,142,154-157]

The VPI AVE7688 was compared to enalapril in a subtotal, or '5/6', nephrectomy model with treatment initiated 21 days after nephrectomy when the animals (8 rats in each treatment group and 5 normal controls) had developed overt proteinuria and hypertension.[138] Both treatments led to reductions in proteinuria but serum creatinine and proteinuria were much lower in animals treated with AVE7688 than with ACEi-alone (enalapril).[138] The beneficial effects were not thought to be mediated through effects on blood pressure as the doses chosen for each drug resulted in similar reductions in blood pressure.[138]

Histology specimens showed AVE7688 greatly reduced the percentage of glomeruli with sclerotic and tubular changes whereas enalapril only partially reduced such changes. AVE7688 increased renal synthesis of NO and decreased synthesis of ET-1 with reduced renal vasoconstriction and increased renal tubular ANP release. The effect on renal NO production was more marked with AVE7688 than enalapril.[138]

Other studies have compared the longer-term effects of omapatrilat with ACEi.[158,159] In one such study, compared with fosinopril or control, omapatrilat led to greater dose-dependent reductions in blood pressure and proteinuria.[158] Both treatments led to similar reductions in GFR, glomerulosclerosis and tubulointerstitial fibrosis compared with control.[158]

In another 5/6 nephrectomy model, treatment was delayed for four weeks' post-surgery allowing the development of hypertension and proteinuria.[159] Following similar initial reductions in proteinuria between omapatrilat (n=6) and enalapril (n=6), omapatrilat resulted in a substantially slower increase in proteinuria than enalapril (despite both drugs having similar effects on blood pressure).[159] Following surgery, control animals (n=15) were euthanized at 12 weeks whilst enalapril-treated rats were euthanized at 32 weeks due to rapidly rising proteinuria. However, omapatrilat-treated rats were not euthanized until 50 weeks at which point levels of proteinuria were similar to pre-treatment levels. On histology, glomerulosclerosis and tubulointerstitial fibrosis scores following 50 weeks of treatment with omapatrilat and 32 weeks of treatment with enalapril were similar to those in control animals at 12 weeks.

In micropuncture studies omapatrilat led to greater reductions in glomerular capillary pressure than enalapril.[159] These findings suggested that combined NEP/ACE inhibition yielded greater renoprotection than ACEi-alone.[159]

Similar effects were observed when omapatrilat was compared with isolated RAS inhibition in models of diabetic nephropathy. These results were very promising given the earlier studies showing NEPi may result in increases in albuminuria.[160,161]

It was hoped that the beneficial renal effects seen in animal studies with combined NEP/RAS inhibition could translate into greater renal protection in people with CKD.[158,159] However, animal studies are often poorly predictive of efficacy in humans, and no studies were performed with VPIs in people with CKD.[162,163]

Some indirect evidence of the renal effects was available from trials of omapatrilat in people with heart failure. The Inhibition of Metallo Protease by Omapatrilat in a

Randomized Exercise and Symptoms Study of Heart Failure (IMPRESS) trial, compared omapatrilat with lisinopril in 573 patients with heart failure.[164] Treatment with omapatrilat was associated with lower rates of elevated creatinine compared with lisinopril (5/289 [1.8%] versus 17/284 [6.1%] respectively; P=0.009), suggesting VPIs provided additional renoprotection over ACEi-alone.[164]

In the Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events (OVERTURE) trial of 5770 patients with heart failure, "renal impairment" occurred less frequently with omapatrilat than enalapril (196/2886 [6.8%] versus 291/2884 [10.1%] respectively) although patients with a serum creatinine greater than 221 µmol/L at baseline were excluded.[165] The effects on renal function occurred despite omapatrilat causing more hypotension than enalapril (564/2886 [19.5%] versus 332/2884 [11.5%]).[165]

The results from these trials supported the animal data suggesting combined NEP/RAS inhibition may have favourable effects on renal function and preservation of GFR over RASi-alone.

3.4.1 Vasopeptidase inhibitors and angioedema

Despite promising effects on kidney function, increasing reports emerged of unacceptable rates of angioedema with omapatrilat, compared with isolated RAS inhibition, in some cases requiring hospitalisation and mechanical ventilation in the most severe cases.[164-166]

The Omapatrilat Cardiovascular Treatment versus Enalapril (OCTAVE) trial, randomized 25,302 hypertensive patients and compared the effects of omapatrilat with enalapril on blood pressure (the primary endpoint) and risk of angioedema (a pre-specified safety event of special interest).[166] At 8 weeks, omapatrilat significantly reduced systolic (3.6 mmHg; P<0.001) and diastolic (2.0 mmHg) blood pressure compared with enalapril. However, across the 24 week treatment period angioedema occurred with greater severity and frequency with omapatrilat than enalapril (274/12,609 [2.17%] versus 86/12,557 [0.68%]; relative risk [RR] 3.17; 95% CI 2.52-4.12; P<0.005).[166] Life-threatening angioedema was rare, with two participants experiencing airway compromise, one of whom required mechanical ventilation.[166] Rates of angioedema were greater in black participants treated with either omapatrilat or enalapril (5.54% and 1.62% respectively) and in current smokers treated with omapatrilat (3.93% versus 0.81% with enalapril).[166]

Following the results of the OCTAVE trial, the Food and Drug Administration (FDA) review board did not approve omapatrilat and it was withdrawn by the manufacturer.[167]

Angioedema is a rare and potentially life-threatening condition caused by a range of aetiologies and is a known side effect of treatment with ACE inhibitors. It is seen in 0.1-0.5% of patients taking ACEi and can occur at any time after initiating treatment with these drugs, although most cases occur within the first week following exposure.[140,168-172] Angioedema commonly causes oedema (without urticaria) of the face, tongue and throat.[172] Very rarely it can cause laryngeal oedema and asphyxiation which may lead to death.[172] Intestinal oedema can manifest with abdominal pain, nausea, vomiting and diarrhoea.[167,172]

ACE inhibitor induced angioedema is believed to be mediated by decreased breakdown of bradykinin resulting in increased bradykinin levels.[167-169,171,173] Bradykinin is a mediator of vascular permeability and vasodilatation and is degraded by ACE (Figure 4).[172,174] In an acute episode of angioedema, bradykinin concentrations can increase more than ten-fold (Figure 4).[140,169,173]

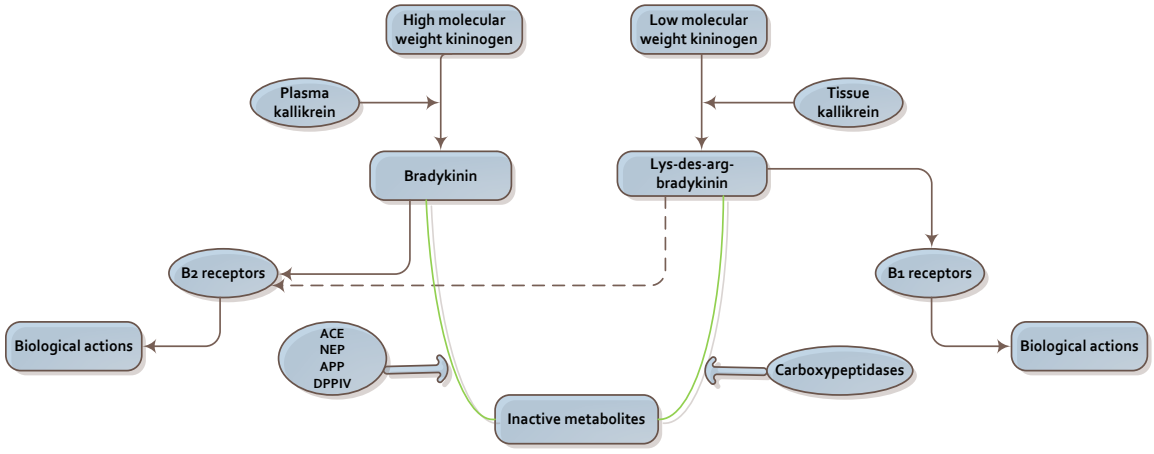


Figure 4: Production and metabolism of bradykinin
ACE = angiotensin converting enzyme; APP = aminopeptidase P; DPPIV = dipeptidyl peptidase IV; NEP = neprilysin

Given the low incidence of angioedema associated with ACEi, it is believed that individuals are more susceptible to developing angioedema if they have an additional predisposing risk factor such as smoking (due to reduced NEP and dipeptidyl peptidase IV [DPPIV] activity in smokers), black race (due to ACE gene polymorphisms) or hereditary angioedema (for example C1 inhibitor deficiency).[174,175] With ACE inhibition, bradykinin degradation becomes dependent on secondary enzymes (including NEP)

for its breakdown (Figure 4) and so combined NEP/RAS inhibition has additive effects on circulating bradykinin concentrations.

Omapatrilat inhibited three key enzymes involved in bradykinin degradation, NEP, ACE and aminopeptidase P (APP), which resulted in substantially higher circulating bradykinin concentrations and increased risk of angioedema compared with isolated ACE inhibition.[167,172,174,176]

ARBs have similar cardiovascular and renal effects to ACEi but with minimal effects on bradykinin activity (as they are not involved in its metabolism) and are therefore much less likely to cause angioedema.[171,173] Since NEPi must be combined with simultaneous RAS blockade, angiotensin receptor-neprilysin inhibitors (ARNI's) were developed, combining the beneficial effects of RAS inhibition (with an ARB) with NEPi, without significantly increasing risk of angioedema (Figure 5).[171,177,178]

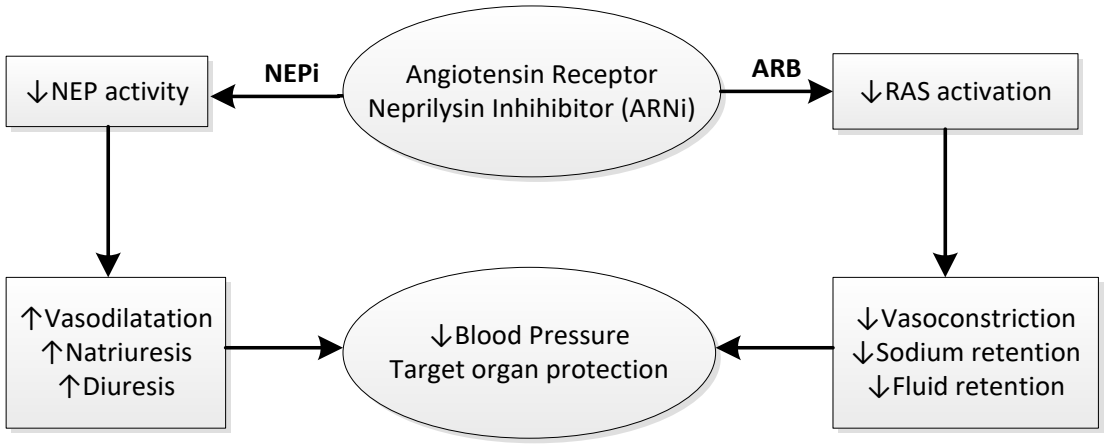


Figure 5: Mechanism of action of angiotensin receptor-neprilysin inhibitors
ARB = angiotensin receptor blocker; NEP = neprilysin; NEPi = neprilysin inhibition; RAS = renin-angiotensin system

4 Angiotensin receptor-neprilysin inhibitor (ARNI)

4.1 Sacubitril/valsartan

Sacubitril/valsartan (previously known as LCZ696) is the first-in-class dual-acting ARNI to be developed. It combines two drugs: an ARB moiety, valsartan, and the NEPi pro-drug sacubitril in a 1:1 molar complex.[178]

Sacubitril/valsartan (trade name Entresto®) received expedited approval from the FDA for treatment of heart failure with reduced ejection fraction (HFrEF) in the US on 7th July 2015[179] and from the UK's National Institute for Clinical Excellence (NICE) in April 2016.[180]

4.2 Pharmacokinetics of sacubitril/valsartan

Following oral administration of sacubitril/valsartan, it is rapidly metabolised delivering systemic exposure to the two separate moieties. Sacubitril (previously known as AHU377), the inactive NEPi, has a relatively short half-life (1.1-3.6 hours) and undergoes further rapid conversion by carboxyl esterases (CES) cleaving an ethyl ester to form the active NEPi, sacubitrilat (previously known as LBQ657).[177,178,181] In *in vitro* studies, this activation primarily occurs in the liver by the hepatic hydrolase CES1.[182] The effect of genetic polymorphisms of CES1 (such as G143E, a loss-of-function variant) on systemic exposure to sacubitrilat is unknown but they are not expected to have a significant impact.[182,183] About 50-70% of sacubitril is excreted in the urine predominantly as sacubitrilat and the remainder in the faeces, whereas valsartan undergoes hepatic elimination (by bile acids) and is excreted mostly unchanged in faeces and only about 10 to 15% in the urine.[184]

In multiple-dosing studies in healthy volunteers, valsartan reached peak plasma concentrations in 1.6-4.9 hours, sacubitril in 0.6-0.9 hours and the active moiety, sacubitrilat, in 1.8-2.7 hours.[178] Sacubitril/valsartan was associated with increases in plasma cGMP, renin and ATII levels, confirming both NEP and RAS inhibition was achieved. Systemic exposure to valsartan following treatment with sacubitril/valsartan demonstrated bioequivalence (e.g., the dose of valsartan in sacubitril/valsartan 97/103

mg is equivalent to 160 mg of valsartan with similar results for other doses of sacubitril/valsartan) and a 40% higher valsartan exposure was achieved than with valsartan-alone.[178]

Pharmacokinetic studies in individuals with renal impairment revealed the steady-state maximum observed concentration (Cmax) increased by ~60% compared with healthy volunteers.[185] The half-life of sacubitrilat increased from 12 hours in healthy volunteers to 21.1, 23.7 and 38.5 hours in those with 'mild' (creatinine clearance [CrCl] 50 to 80 ml/min [n=8]), 'moderate' (CrCl 30 to 50 ml/min [n=8]) and 'severe' renal impairment (CrCl less than 30 ml/min [n=6]) respectively.[185]

A lower starting dose of sacubitril/valsartan 24/26 mg has been recommended in people with an eGFR of 30 to 60 mL/min/1.73m^2 and, for an eGFR less than 30 mL/min/1.73m^2 caution has been advised due to limited exposure in this population.[186] No clinically relevant pharmacokinetic drug-drug interactions have been observed between sacubitril/valsartan and a variety of drug classes.[187,188]

Several clinical trials including people with hypertension and heart failure have assessed the safety and efficacy of sacubitril/valsartan in these populations.

4.3 Effects of sacubitril/valsartan on blood pressure

In spontaneously hypertensive rats, sacubitril/valsartan demonstrated superior blood pressure lowering (irrespective of dietary salt consumption) compared with valsartan-alone or vehicle and ameliorated cardiac hypertrophy, fibrosis, coronary vascular remodelling and endothelial dysfunction.[189]

A trial of 1328 people with mild-to-moderate hypertension, compared sacubitril/valsartan over 8 weeks with valsartan-alone, sacubitril-alone or placebo. Sacubitril/valsartan had superior diastolic blood pressure lowering (primary outcome mean diastolic blood pressure reduction 2.17 mmHg; 95% CI 1.06-3.28; P<0.0001) compared with valsartan.[190] Mean sitting systolic blood pressure was 4.20 (-5.94 to -2.46; p<0.0001) mmHg lower with sacubitril/valsartan.

In single-dose pairwise comparisons, each sacubitril/valsartan dose had greater blood pressure lowering than the equivalent dose of valsartan and, the proportional reduction in systolic and diastolic blood pressure was greater with increasing sacubitril/valsartan

doses (Figure 6). Mean sitting (and ambulatory) pulse pressure was 3.32 (95% CI -5.51 to -1.13) mmHg lower in people allocated sacubitril/valsartan (2 tablets of 97/103 mg daily) versus valsartan (320 mg daily).[190] Sacubitril/valsartan was generally well-tolerated and no cases or angioedema or death occurred.[190]

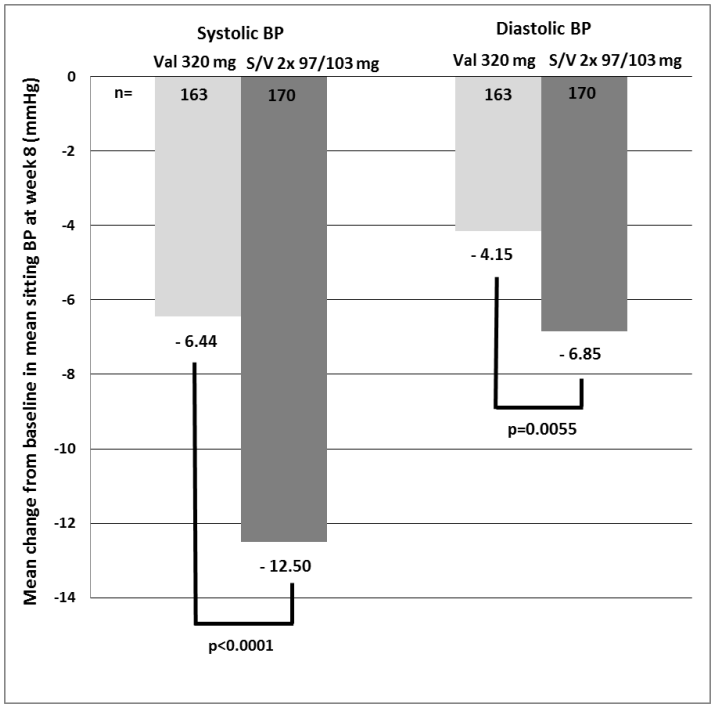


Figure 6: Difference in mean sitting systolic and diastolic blood pressure (BP) at 8 weeks with maximum sacubitril/valsartan (S/V; n=170) dose compared with full dose valsartan (n=163)
Adapted from Ruilope LM, et al. Lancet 2000[190]. S/V = sacubitril/valsartan; Val = valsartan

Similar blood pressure effects have been reported in Asian patients (from Japan, China, Korea, Taiwan and Thailand) with hypertension, who are generally less responsive to isolated RASi as they often have higher salt-intake and are more prone to SSH.[191]

The Prospective Comparison of Angiotensin Receptor Neprilysin Inhibitor with Angiotensin Receptor Blocker Measuring Arterial Stiffness in the Elderly (PARAMETER) trial, randomized 454 people aged over 60 years with systolic hypertension and pulse pressure greater than 60 mmHg (suggestive of arterial stiffness), to sacubitril/valsartan or olmesartan.[192] The primary outcome assessed the effect of sacubitril/valsartan on central aortic pressures at 12 weeks.[192] Sacubitril/valsartan, compared with olmesartan, was associated with greater reductions in central aortic systolic pressure (12.6 [95% CI -14 to -10.1] mmHg versus 8.9 [95% CI -11.1 to -6.7] mmHg respectively), central aortic pulse pressure (6.4 [95% CI -7.7 to

-5.1] mmHg versus 4.0 [95% CI -5.3 to -2.6] mmHg respectively; P=0.01) and central mean arterial pressure (8.5 [95% CI -10.0 to -7.1] mmHg versus 6.5 [95% CI -8.0 to -5.0] mmHg respectively) following 12 weeks of treatment.[192]

The Effect of Sacubitril-Valsartan vs Enalapril on Aortic Stiffness in Patients With Heart Failure and Reduced Ejection Fraction (EVALUATE-HF) trial randomized 464 people with HFrEF sacubitril/valsartan (n=231) or enalapril (n=233).[193] The primary outcome was change in aortic characteristic impedance (Zc; a measure of central aortic stiffness) from baseline to 12 weeks.[193]

Between baseline and 12 weeks, there was no significant between-group difference in Zc in those allocated sacubitril/valsartan compared with enalapril (-2.2 [95% CI -17.6 to 13.2] dyne×sec/cm^5; P=0.78), despite a 6.4 mmHg reduction in brachial systolic blood pressure with sacubitril/valsartan compared with only 1.6 mmHg with enalapril (between-group difference -4.8 [95% CI -7.6 to -2.1] mmHg; P=0.001).[193]

In 8442 patients with HFrEF, at 8 months mean systolic blood pressure was 3.2±0.4 mmHg lower in patients allocated sacubitril/valsartan compared with enalapril (P<0.001).[194] Overall throughout the trial a mean reduction in systolic blood pressure of 2.70 (95% CI -3.07 to -2.34) mmHg was achieved with sacubitril/valsartan compared with enalapril.[194]

In a trial of 301 people with heart failure with preserved ejection fraction (HFpEF), at 12 weeks sacubitril/valsartan reduced systolic and diastolic blood pressure by 9.3 (SD 14) mmHg and 4.9 (SD 10) mmHg respectively, compared with 2.9 (SD 17) mmHg and 2.1 (SD 11) mmHg in people allocated valsartan.[195] In a much larger trial of 4822 patients with HFpEF, at 8 months sacubitril/valsartan reduced mean systolic blood pressure by 4.5 mmHg (95% CI 3.6 to 5.4) compared with valsartan.[196]

In heart failure trials, the greater blood pressure lowering effect of sacubitril/valsartan has not been shown to correlate with the treatment effect. Whether such reductions in blood pressure translate into improved renal and CV outcomes in patients with CKD or hypertension remains unclear. Apart from in heart failure populations, no large-scale randomized clinical outcome trials have been performed with sacubitril/valsartan for hypertension or other conditions.

4.4 Renal effects of sacubitril/valsartan

4.4.1 Results from in-vitro and animal studies

In-vitro studies have shown that addition of sacubitrilat to human adrenocortical cells enhanced the ability of ANP and BNP to block aldosterone synthesis.[197] In another in-vitro study, addition of sacubitrilat and valsartan (mimicking the ARNI sacubitril/valsartan) to cultured renal mesangial cells improved the inhibitory effect of valsartan on collagen synthesis in renal mesangial cells.[198]

In a rodent model of diabetic nephropathy, rats were treated at 2 or 8 weeks after the onset of diabetes with irbesartan (n=8), irbesartan plus NEPi (thiorphan, n=16) or vehicle-alone (n=8).[199] Irbesartan combined with thiorphan reduced proteinuria (with levels normalising at 12 weeks), albuminuria and glomerulosclerosis to a greater extent than irbesartan-alone, despite similar reductions in blood pressure.[199]

4.4.2 Results from clinical trials of sacubitril/valsartan

4.4.2.1 *Effects on renal function*

Data on the effects of ARNI in people with CKD have mostly been extrapolated from individuals with an eGFR of 30 to 60 mL/min/1.73m^2 participating in trials of heart failure or hypertension. Data on the effects of NEPi with sacubitril/valsartan in people with CKD, particularly advanced CKD (stage 4 or 5), was scarce since such individuals were not included in randomized trials of sacubitril/valsartan.

The Prospective comparison of ARNi with ARB on Management Of heart failure with preserved ejection fraction (PARAMOUNT) trial randomized 301 patients with HFpEF to maximum tolerated daily doses of sacubitril/valsartan (97/103 mg twice daily [n=149]) or valsartan-alone (320 mg once daily [n=152]).[195] 42% of PARAMOUNT participants had an eGFR less than 60 mL/min/1.73m² at baseline (56/149 [38%] in the sacubitril/valsartan arm and 69/152 [45%] in the valsartan arm) and patients with an eGFR less than 30 mL/min/1.73^2 were excluded.[195] Mean (SD) eGFR at baseline in those allocated sacubitril/valsartan 67 (19.4) mL/min/1.73m² and 64 (21.3) mL/min/1.73m² in those allocated valsartan.[195]

At the end of the initial 12-week treatment period, mean eGFR did not differ significantly between the treatment groups (between-group difference 1.8 mL/min/1.73m^2; P=0.14). However, at 36 weeks, there was a smaller decline in eGFR in participants randomized

to sacubitril/valsartan than valsartan-alone (1.5±13.1 versus 5.2±11.4 mL/min/1.73m² respectively; P=0.008) compared with baseline.[200]

The Prospective comparison of ARNI with ACEi to Determine Impact on Global Mortality and morbidity in Heart Failure trial (PARADIGM-HF) trial, randomized 8442 patients with HFrEF to maximum doses of sacubitril/valsartan (n=4187) or enalapril (n=4212).[181,201] Baseline eGFR was 67.7 mL/min/1.73m^2, and 36% (1541/4187 [37%] allocated sacubitril/valsartan and 1520/4212 [36%] allocated enalapril) of participants had an eGFR less than 60 ml/min/1.73m^2.[167,201] Amongst those with CKD, eGFR was 49±8 mL/min/1.73m^2 at screening.[202] The composite renal endpoint, "decline in renal function" included time to; i) 50% decline in eGFR relative to baseline eGFR; ii) more than 30 mL/min/1.73m^2 decline in eGFR from baseline to less than 60 mL/min/1.73m^2 or iii) progression to ESKD.[181,201]

Between screening and the end of follow-up, eGFR declined less in people allocated sacubitril/valsartan compared with enalapril (7.8 [95% CI 9.6-6.0] versus 10.2 [95% CI 12.1-8.3] mL/min/1.73m^2 respectively).[202] The annual rate of decline in eGFR was slower with sacubitril/valsartan compared with enalapril (1.61 [95% CI -1.77 to -1.44] versus 2.04 [95% CI -2.21 to-1.88] mL/min/1.73m^2/year respectively; P<0.001) with no heterogeneity in the treatment effect by CKD status at screening (P for interaction = 0.54).[202]

PARADIGM-HF participants with diabetes had a faster rate of decline than those without diabetes (2.0 [95% CI 1.9-2.1] versus 1.1 [95% CI 1.1-1.2] mL/min/1.73m^2 respectively; P<0.0001).[203] The beneficial effect of sacubitril/valsartan in slowing the annual rate of change in eGFR compared with enalapril, was marginally greater amongst patients with diabetes than those without diabetes (difference 0.6 mL/min/1.73m²/year [95% CI 0.4-0.8] versus 0.3 mL/min/1.73m²/year [0.2-0.5] respectively; P for interaction = 0.038).[203]

There was a non-significant trend towards lower rates of the composite renal endpoint in patients treated with sacubitril/valsartan than enalapril (94/4187 [2.2%] versus 108/4212 [2.6%] respectively; HR 0.86 [95% CI 0.65-1.13]; P=0.28), despite greater reductions in blood pressure with sacubitril/valsartan.[194] A post-hoc analysis using an alternative definition for the composite renal outcome of ESKD or a 50% or greater decline in eGFR from baseline, suggested sacubitril/valsartan, compared with enalapril, substantially reduced this adverse renal outcome (37/4187 [0.9%] versus 58/4212 [1.4%] respectively; HR 0.63 [95% CI 0.42-0.95]; P=0.028) with no heterogeneity in the effect by CKD status at baseline (P for interaction = 0.97).[202]

Progression to ESKD occurred less frequently with sacubitril/valsartan than enalapril (8/4187 [0.2%] versus 16/4212 [0.4%] respectively; P=0.11).[194] However, the number of ESKD events was small as the trial did not include participants with advanced CKD (eGFR less than 30 mL/min/1.73m^2) who would be much more likely to progress to ESKD, the trial therefore lacked statistical power to assess this outcome.

The Prospective Comparison of ARNI with ARB Global Outcomes in HF with Preserved Ejection Fraction (PARAGON-HF) trial, randomized 4822 patients with HFpEF to sacubitril-valsartan 97/103 mg twice daily or valsartan 160 mg twice daily.[196] Mean eGFR was 62.5±19 mL/min/1.73m^2 at baseline and 48.5% (2341/4822) had an eGFR of 30-60 mL/min/1.73m^2 (patients with an eGFR less than 30 mL/min/1.73m^2 were excluded).[196]

Risk of the secondary renal composite outcome (defined as: [i] death from renal failure; [ii] ESKD or; [iii] decline in eGFR of 50% or more from baseline) was halved in patients randomized to sacubitril/valsartan compared with valsartan-alone (33/2407 [1.4%] versus 64/2389 [2.7%]; HR 0.50 [95% CI 0.33-0.77]).[196]

A tabular meta-analysis assessing the effects of combined NEP/RAS inhibition on “decline in renal function” in patients with heart failure, suggested this treatment combination may be associated with a 32% (risk ratio 0.68; 95% CI 0.51-0.92; P=0.01) reduction in the risk of decline in renal function compared with isolated RAS inhibition.[204] However, there was significant heterogeneity between the trial populations, study designs and definitions of the renal outcome between the trials (overall P for heterogeneity = 0.10).[204] The total number of renal events was also small (298 events in 7511 participants allocated combined NEP/RAS inhibition versus 423 events in 7532 participants allocated isolated RAS inhibition) and the majority of events were contributed by just two trials (PARADIGM-HF and OVERTURE).[204]

It is hypothesised that the lack of expected decline in GFR despite significant reductions in systemic blood pressure in the heart failure trials amongst participants allocated ARNIs, may result from afferent arteriolar vasodilation with only a relative efferent arteriolar vasoconstriction (due to actions of NPs at this site). This increase in intraglomerular capillary hydrostatic pressure enabled GFR to be maintained despite low systemic pressure.[205]

The data from the heart failure and hypertension trials suggested that combined NEP/RAS inhibition may have a beneficial effect on kidney function, however significant

uncertainty remained as to the true effects especially in people with advanced CKD, in whom this treatment strategy had not been studied.

4.4.2.2 *Effects on albuminuria*

In 1328 patients with mild-moderate hypertension, sacubitril/valsartan reduced albuminuria more than placebo, but not more than the equivalent dose of valsartan.[190] However, baseline urine albumin:creatinine ratio (uACR) was low (geometric mean between 1.1 and 1.5 mg/mmol) in all treatment groups.[190] Compared with baseline, at 8 weeks, sacubitril/valsartan 194/206 mg reduced uACR by 12% (95% CI -25 to 4%; P<0.05) and valsartan 320 mg reduced uACR by 10% (95% CI -24 to 8%; P<0.05), compared with placebo.[190]

In contrast to people with hypertension, albuminuria *increased* in patients with heart failure following treatment with sacubitril/valsartan, although uACR was very low at baseline in all treatment groups. In PARAMOUNT, at 36-weeks geometric mean uACR increased by 0.5 mg/mmol with sacubitril/valsartan (from 2.4 mg/mmol to 2.9 mg/mmol) but remained stable with valsartan (from 2.1 mg/mmol to 2.0 mg/mmol), P for difference=0.016.[200]

In PARADIGM-HF, uACR was measured in 22% of participants (1872/8442) at screening and was re-measured at randomization, one month and eight months post-randomization.[202] Median (interquartile range [IQR]) uACR at screening was 1.0 (0.4-43.2) mg/mmol and 24% of participants had micro- or macroalbuminuria (median uACR in participants with albuminuria 7.55 mg/mmol; 95% CI 2.55-21.8).[202] Following randomisation, uACR increased by 0.30 (95% CI 0.10-0.50) mg/mmol with sacubitril/valsartan and by a similar amount with enalapril.[206] At one-month post randomization, uACR returned back down to screening levels with enalapril but remained significantly higher in those allocated sacubitril/valsartan even at 8 months.[202,206]

The effects of NPs on glomerular haemodynamics that results in increased intraglomerular capillary hydrostatic pressure and glomerular permeability may explain the rise in albuminuria seen in people with heart failure.[205] The effects of sacubitril/valsartan on albuminuria in people with heart failure raises uncertainty as to the effects of the drug in CKD populations. If sacubitril/valsartan increased albuminuria in people with CKD (who often have much higher levels of albuminuria) this would be of significant concern given the established link between albuminuria and progression of CKD.[4,29]

4.5 Cardiovascular effects of sacubitril/valsartan

In early heart failure, NP levels increase to counteract salt and water retention. Over time the effects of NPs are negated by up-regulation of neurohormonal pathways (including RAS and the sympathetic nervous system) which causes further salt and water retention. In animal models of cardiac disease, sacubitril/valsartan improved cardiac dysfunction and remodelling compared with isolated NEP or RAS inhibition.[207,208]

4.5.1 Effects on cardiac biomarkers

In heart failure, NT-proBNP is an important and useful prognostic biomarker as it is not degraded by NEP (unlike BNP), so changes in NT-proBNP concentrations can be used to assess disease severity in patients treated with NEPi.[177,195]

The Comparison of Sacubitril/Valsartan versus Enalapril on Effect on NT-proBNP in Patients Stabilized from an Acute Heart Failure Episode (PIONEER-HF) trial, randomized 881 patients with acute decompensated heart failure due to HFrEF, to sacubitril/valsartan (n=440) or enalapril (n=441).[209] Sacubitril/valsartan was associated with a significant 46.7% time-averaged reduction in geometric mean NT-proBNP concentration (at 4 and 8 weeks versus baseline) compared with a 25.3% reduction with enalapril (ratio of change 0.71; 95% CI 0.63-0.81; P<0.001).[209] There was no heterogeneity in the treatment effect of sacubitril/valsartan on NT-proBNP by eGFR at baseline (P for interaction = 0.81) or by any other subgroup.[209]

Sacubitril/valsartan reduced mean concentrations of high-sensitivity troponin T (hs-TnT) by 16% (P<0.001) and soluble suppression of tumourigenicity-2 (sST2; a marker of cardiac stress, stretch and fibrosis and a prognostic marker in ADHF) by 9% (P=0.0035) and this reduction emerged by 4 weeks, compared with enalapril.[210] Exploratory analyses suggested sacubitril/valsartan reduced heart failure re-admission rates compared with enalapril (35/440 [8.0%] versus 61/441 [13.8%] respectively; HR 0.56 [95% CI 0.37-0.84]).[209]

The PARAMOUNT trial assessed the effects of sacubitril/valsartan on NT-proBNP (as a marker of LV wall stress) and troponin-T (as a marker of cardiac myocyte damage) concentrations.[195] At 12 weeks, sacubitril/valsartan resulted in a significant reduction in NT-proBNP (the primary outcome) concentration compared with valsartan (ratio of change 0.77 [95% CI 0.64-0.92]; P=0.005) but, the effect was not maintained at 36

weeks (0.85; 95% CI 0.65-1.09; P=0·20).[195] Ratio of change in hs-TnT between sacubitril/valsartan and valsartan was only marginally significant at 12 weeks post-randomization (0.88; 95% CI 0.77-1.00; P=0.05) compared with 36 weeks (0.86; 95% CI 0.75-0.99; P=0.03).[211]

The change in NT-proBNP concentrations between the two groups remained significant at 12 weeks even after adjustment for blood pressure,[195,212] suggesting the effects of sacubitril/valsartan on cardiac biomarkers may be independent of any blood pressure lowering effects.[197,217] There was also no significant heterogeneity in the treatment effect on NT-proBNP between individuals with an eGFR greater or less than 60 mL/min/1.73m^2 (P=0.18).[195]

Similar results were seen in PARADIGM-HF patients with HFrEF in whom NT-proBNP concentrations were significantly lower at 1 and 8 months post-randomization in people treated with sacubitril/valsartan compared with enalapril (P<0.0001 for difference between the groups at both time points).[213] Sacubitril/valsartan did not affect concentrations of other cardiac biomarkers (including sS2 or galactin-3 [involved in tissue repair, cardiac remodelling, and fibrosis in heart failure]).[214]

In EVALUATE-HF, sacubitril/valsartan compared with enalapril, produced greater reductions between baseline and 12 weeks in concentrations of NT-proBNP (ratio of change 0.67; 95% CI 0.59-0.76; P<0.001), hs-TnT (ratio of change 0.83; 95% CI 0.78-0.88; P<0.001) and, sST-2 (ratio of change 0.94; 96% CI 0.89-0.98; P=0.006).[193] Post-hoc analyses suggested changes in NT-proBNP concentrations may correlate with changes in improvements in Kansas City Cardiomyopathy Questionnaire (KCCQ) summary score and LV end diastolic volume index on echocardiography.[193]

The data from cardiac biomarker studies suggest that sacubitril/valsartan has beneficial effects on cardiac structure and function compared with isolated RAS inhibition. Given the manifestation of CVD in CKD (with heart failure and sudden cardiac in advanced CKD)[88] the data from heart failure populations suggest treatment with sacubitril/valsartan could potentially provide a similar degree of benefit in people with CKD in reducing the burden of cardiovascular complications in this population, as seen in people with heart failure and hypertension.

4.5.2 Effects on cardiovascular outcomes

In early heart failure, NP levels increase to counteract salt and water retention. Over time the effects of NPs are negated by up-regulation of neurohormonal pathways (including RAS and the sympathetic nervous system) which causes further salt and water retention. In animal models of cardiac disease, sacubitril/valsartan improved cardiac dysfunction and remodelling compared with isolated NEP or RAS inhibition.[207,208]

In the PARADIGM-HF trial sacubitril/valsartan, compared with enalapril, produced a highly significant 20% (914/4187 [21.8%] versus 1117/4212 [26.5%] respectively; HR 0.80 [95% CI 0.73-0.87]; P<0.001) reduction in the primary endpoint of death from cardiovascular causes or hospitalisation for heart failure.[194] The individual components of the primary endpoint were reduced by a similar proportion with a 20% reduction in cardiovascular mortality (558/4187 [13.3%] versus 693/4212 [16.5%]; HR 0.80 [95% CI 0.71-0.89]; P<0.001) and a 21% reduction in hospitalisation for worsening heart failure (537/4187 [12.8%] versus 658/4212 [15.6%]; HR 0.79 [95% CI 0.71-0.89]; P<0.001).[194]

Sacubitril/valsartan reduced all-cause mortality by 16% compared with enalapril (711/4187 [17.0%] versus 835/4212 [19.8] respectively; HR 0.84 [95% CI 0.76-0.93]; P<0.001). There was no heterogeneity in the treatment effect with sacubitril/valsartan on the primary outcome in a range of baseline subgroups including: eGFR above or below 60 mL/min/min/1.73m^2 (P value for interaction = 0.91), presence or absence of diabetes mellitus (P value for interaction = 0.40), systolic blood pressure above or below the trial median value (P value for interaction = 0.87).[194]

There were 1546 deaths in PARADIGM-HF: 711 deaths amongst participants allocated sacubitril/valsartan and 835 amongst those allocated enalapril.[215] 80.9% (1251/1546) of deaths were due to cardiovascular aetiologies; 13.3% amongst participants allocated sacubitril/valsartan and 16.5% amongst participants allocated enalapril.[215] 44.8% of cardiovascular deaths were due to sudden cardiac death (defined as an unexpected death in an apparently stable patient last seen 24 hours prior to death) and 26.5% were due to worsening heart failure.[215] Treatment with sacubitril/valsartan, compared with enalapril, reduced the risk of sudden cardiac death by 20% (HR 0.80; 95% CI 0.68-0.94; P=0.008) and death due to worsening heart failure by 21% (HR 0.79; 95% CI 0.64-0.98; P=0.034).[215]

Analyses of the effect of sacubitril/valsartan across a range of systolic blood pressure values (less than 110 mmHg and above 140 mmHg) demonstrated a consistent risk reduction in the primary outcome even in people with extremely low blood pressure (a

population of patients with heart failure at particularly high risk of adverse outcomes), compared with enalapril.[216] The precise mechanism by which sacubitril/valsartan, influences cardiovascular mortality is unknown although suggested mechanisms include; counter-regulation of RAS and sympathetic nervous systems, upregulation of NP levels and reduced myocardial fibrosis and remodelling.[215]

In PARAGON-HF allocation to sacubitril/valsartan, compared with valsartan, numerically reduced the number of events for each of the components of the primary outcome of the trial (a composite of total hospitalizations for heart failure and cardiovascular mortality), but these changes were not statistically significantly (894 events in 526/2407 versus 1009 events in 557/2389 respectively; Rate Ratio 0.87 [95% CI 0.75-1.01]; P=0.06).[196] Sacubitril/valsartan (compared with valsartan) had no effect on cardiovascular mortality (204/2407 [8.5%] versus 212/2389 [8.9%] respectively; HR 0.95 [95% CI 0.79-1.16]) or all-cause mortality (342/2407 [14.2%] versus 349/2389 [14.6%] respectively; HR 0.97 [95% CI 0.84-1.13]).[196]

Sacubitril/valsartan was associated with greater improvements in NYHA class from baseline to 8 months, compared with valsartan (347/2316 [15%] versus 289/2302 [12.6] respectively; Odds Ratio [OR] 1.45 [95% CI 1.13-1.86).[196] At 8 months, the mean change in KCCQ clinical summary score was 1.0 (95% CI 0.0-2.1) point higher in participants allocated sacubitril/valsartan, suggesting reduced symptoms and physical limitations compared with valsartan.[196]

The similarities in the manifestation of CVD observed in patients with advanced CKD and patients with HF raises the hypothesis that treatments proven to be effective in heart failure may prove beneficial in CKD populations.

4.6 Safety concerns with sacubitril/valsartan

4.6.1 Angioedema

A major safety concern with ARNIs has been of risk of developing angioedema. In PARAMOUNT, only one case of angioedema occurred in a patient taking sacubitril/valsartan but they were not admitted to hospital.[195] In the EVALUATE-HF trial only one case of angioedema was reported in a patient allocated enalapril. In PIONEER-HF seven cases of angioedema occurred; one case in a participant allocated

sacubitril/valsartan and six cases in participants (all of black ethnicity) allocated enalapril (RR 0.17; 95% CI 0.02-1.38).[193,209,217]

In PARADIGM-HF (the largest trial of sacubitril/valsartan) angioedema rates did not differ significantly with sacubitril/valsartan compared with enalapril, and no cases of life-threatening angioedema with airway compromise occurred (Table 3).

Angioedema severity	Sacubitril/valsartan (n=4187) (%)	Enalapril (n=4212) (%)	P value
No treatment or treated only with antihistamines	10 (0.2)	5 (0.1)	0.19
Treatment with glucocorticoids or catecholamines without admission to hospital	6 (0.1)	4 (0.1)	0.52
Admitted to hospital but no evidence of airway compromise	3 (0.1)	1 (<0.1)	0.31

Table 3: Angioedema rates in PARADIGM-HF patients treated with sacubitril/valsartan and enalapril
Table adapted from McMurray, et al. *NEJM* 2014.[194]

In PARAGON-HF, sacubitril/valsartan was associated with a significant 0.4% absolute excess in cases of angioedema without airway compromise, compared with valsartan (14/2407 [0.6%] versus 4/2389 [0.2%]; P=0.02).[196]

4.6.2 Renal safety

In PARAMOUNT, rates of renal serious adverse events were similar between sacubitril/valsartan and valsartan (2/149 [1%] versus 3/152 [2%]; P=0.98).[195] Overall rates of all adverse event reports of 'renal dysfunction' (3/149 [2%] versus 7/152 [5%]; P=0.34) and 'hyperkalaemia' (12/149 [8%] versus 9/152 [6%]; P=0.50) were similar in those allocated sacubitril/valsartan and valsartan respectively.[195]

Rates of the more specific renal adverse event of 'worsening renal function' (defined as an increase in serum creatinine of more than 26.5 μmol/l [0.3 g/dL] and/or an increase of more than 25% between two time-points), severe hyperkalaemia or declines in eGFR did not differ between sacubitril/valsartan and valsartan (Table 4).[195,200]

Adverse event (AE)	Sacubitril/valsartan (n=149) (%)	Valsartan (n=152) (%)	P value
Worsening renal function			
12 weeks	6 (5)	9 (7)	0.68
36 weeks	7 (6)	16 (13)	0.08
Anytime during the trial	16 (12)	25 (18)	0.28
≥50% decline in eGFR	5 (3)	4 (3)	0.98
Potassium ≥6.0 mmol/L	5 (3)	6 (4)	0.97

Table 4: Renal safety data from PARAMOUNT with sacubitril/valsartan and valsartan-alone
Adapted from Solomon, et al. *Lancet* 2012.[195]

In the PARADIGM-HF and PARAGON-HF trials, rates of hyperkalaemia occurred less frequently with sacubitril/valsartan than enalapril or valsartan respectively (Table 5).[194,196] In PARADIGM-HF, sacubitril/valsartan was associated with significantly fewer rises in serum creatinine above 221 µmol/L than with enalapril and, there were fewer discontinuations of sacubitril/valsartan for renal impairment compared with enalapril (10.7% versus 12.3% respectively; P=0.03).[194]

Rates of hyperkalaemia (potassium greater than 5.5 mmol/L) were much lower amongst participants allocated sacubitril/valsartan in both PARADIGM-HF and PARAGON-HF (Table 5). In PARADIGM-HF participants taking mineralocorticoid receptor antagonists at baseline, allocation to sacubitril/valsartan was associated with a lower incidence of severe hyperkalaemia (serum potassium above 6.0 mmol/L) compared with enalapril (2.2 versus 3.1 per 100 patient-years respectively; HR 1.37 [95% CI 1.06-1.76]; P = 0.02).[218]

	PARADIGM-HF			PARAGON-HF		
Adverse event	Sacubitril/valsartan (n=4187) (%)	Enalapril (n=4212) (%)	P value	Sacubitril/valsartan (n=2407) (%)	Valsartan (n=2389) (%)	P value
*K >5.5 mmol/L	674 (16.1)	727 (17.3)	0.15	316/2386 (13.2)	361/2367 (15.3)	0.048
*K >6.0 mmol/L	181 (4.3)	236 (5.6)	0.007	75/2386 (3.1)	101/2367 (4.3)	0.04
†SCr ≥221 µmol/L	139 (3.3)	188 (4.5)	0.007	97 (4.0)	109 (4.6)	0.36
†SCr ≥283 µmol/L	63 (1.5)	82 (2.0)	0.10	38 (1.6)	40 (1.7)	0.79

Table 5: Renal adverse events following treatment with sacubitril/valsartan in patients with heart failure
Adapted from McMurray JJ, et al. *NEJM* 2014[194] and Solomon SD, et al. *NEJM* 2019[196]
*K = potassium. †SCR = serum creatinine

In PIONEER-HF, there was no difference in rates of worsening renal failure (defined as fall in eGFR of 25% or more and a rise in serum creatinine of 44 micromol/L or higher) between sacubitril/valsartan compared with enalapril (60/440 [13.6] versus 65/441 [14.7%] respectively; RR 0.93 [95% CI 0.67 to 1.28]).[209] Sacubitril/valsartan, was associated with numerically, but not statistically, higher rates of hyperkalaemia (51/440 [11.6%] versus 41/441 [9.3%] respectively; RR 1.25 [95% CI 0.84-1.84]) and symptomatic hypotension (66/440 [15.0%] versus 56/441 [12.7%] respectively; RR 1.18 [95% CI 0.85-0.64]).[209]

The rate at which sacubitril/valsartan is titrated to maximum treatment doses has not been shown to affect rates of renal dysfunction, hyperkalaemia or symptomatic hypotension.[217]

4.6.3 Hypotension

In PARADIGM-HF, symptomatic hypotension occurred more frequently with sacubitril/valsartan than enalapril (588/4187 [14%] versus 388/4212 [9.2%]; P<0.001), as did symptomatic hypotension with systolic blood pressure of less than 90 mmHg (112/4187 [2.7] versus 59/4212 [1.4%]; P <0.001). Similarly in PARAGON-HF, sacubitril/valsartan was associated with a 5% excess in cases of hypotension with a systolic blood pressure less than 100 mmHg compared with valsartan (380/2407 [15.8%] versus 257/2389 [10.8%]; P<0.001).[196]

Despite the hypotension, fewer participants allocated sacubitril/valsartan, compared with enalapril, discontinued study treatment because of adverse events in PARADIGM-HF (448/4187 [10.7%] versus 518/4212 [12.3%] respectively; P=0.03) and in PARAGON-HF, compared with valsartan (371/2407 [15.4%] versus [387/2389 [16.2%] respectively).[197,199,216] Importantly, a lower blood pressure associated with sacubitril/valsartan did not adversely affect renal function in either trial and in PARADIGM-HF sacubitril/valsartan was associated with fewer withdrawals of study treatment due to renal impairment (0.7% versus 1.4% with enalapril; P=0.002).[194,196,213]

4.6.4 Liver safety

Rates of liver-related adverse events did not differ between sacubitril/valsartan and valsartan in PARAGON-HF (151/2407 [6.3%] versus 178/2389 [7.5%] respectively; P=0.11).[196] No significant liver-related adverse events were reported in other heart failure trials of sacubitril/valsartan.[192,194,195]

4.6.5 Cognition

There is a theoretical concern that NEP inhibition may affect cognition and lead to the development of dementia, as NEP is one of the enzymes involved in the breakdown of β-amyloid (Aβ).[219] In Alzheimer's dementia (AD) and cerebral amyloid angiopathy (CAA) there is an accumulation of Aβ peptides in the brain.[220] Several other amyloid-degrading enzymes are involved in the degradation of Aβ (including; ACE, ECE-1, NEP2 and insulin degrading enzyme) in addition to amyloid transporters (such as apolipoprotein E).[221] In order for NEP inhibitors to influence Aβ accumulation in the brain, the drugs would need to cross the blood-brain barrier. Accumulation of Aβ occurs over many years before any clinical manifestations of dementia occur, so studies in humans to assess the potential effects of NEP inhibition on the brain would require long-term follow-up.

Due to concerns regarding cognition, studies (a short-term study lasting 2 weeks and a long-term study over 39 weeks) were undertaken in cynomolgus monkeys (which have greater evolutionarily resemblance to humans than other animals) to assess whether sacubitrilat crossed the blood-brain barrier and what, if any, effect sacubitril/valsartan had on cerebrospinal fluid concentrations of Aβ isoforms A.[222] Despite very low levels of sacubitril/valsartan in the cerebrospinal fluid compared with plasma, sufficient NEP inhibition occurred in the cerebrospinal fluid resulting in a rapid rise in Aβ isoforms 1-40, 1-38 and Aβ total due to impaired clearance.[222] There was no change in brain levels of Aβ isoforms and brain histology showed no treatment-related increases in Aβ deposition or plaque formation with the brain or cerebral vasculature.[222]

A study similar to that undertaken in monkeys was performed in healthy human volunteers.[223] The study randomized healthy volunteers to sacubitril/valsartan (n=21) or matching placebo (n=22) for 14 days and undertook serial cerebrospinal fluid sampling.[223] Sacubitril/valsartan did not significantly affect levels of Aβ isoforms 1-42 and 1-40 (the two isoforms found in amyloid plaques within the brains of patients affected with Alzheimer's) compared with placebo but levels of Aβ 1-38 were significantly elevated.[223] The clinical relevance of a rise in Aβ 1-38 in the brain or its effect on cognitive decline is unknown.

Due to these concerns, the PARADIGM-HF investigators undertook a detailed review of Medical Dictionary for Regulatory Activities (MedDRA) terms reported. Using "broad" and "narrow" preferred terms (PTs), no increase in cognition, memory or dementia-related adverse events with sacubitril/valsartan compared with enalapril was found.[224] The age-adjusted annual rate of broad PT adverse events reports in participants

assigned sacubitril/valsartan was 0.95 (95% CI 0.75-1.15) per 100 patient-years group compared with 0.98 (95% CI 0.77–1.19) per 100 patient-years in participants assigned enalapril.[224]

A more detailed sub-study examining the effects of sacubitril/valsartan on cognitive function was undertaken in the PARAGON-HF trial. Change in mini mental state examination [MMSE] at 2 years was assessed, and the results of this sub-study are awaited.[225]

4.6.6 Metabolic effects

In vitro studies showed NPs stimulated lipolysis in adipocytes and, in patients with heart failure infusions, of ANP led to increased circulating levels of free fatty acids.[226] Excess lipid mobilization could potentially cause ectopic fat storage in muscles and in the liver resulting in insulin resistance, as seen in type 2 diabetes mellitus.[226]

In a large Mendelian randomization study among people without type 2 diabetes mellitus or cardiovascular disease, an inverse relationship between levels of NT-proBNP and risk of type 2 diabetes mellitus was shown, suggesting BNP may have a protective role in this disease.[227]

In animals with SSH, sacubitril/valsartan, compared with valsartan, did not have any effects on insulin, glucose or lipids.[189] In a study of 98 people with obesity and hypertension, 8 weeks of treatment with sacubitril/valsartan was associated with improved insulin sensitivity without significant effects on fasting levels of serum free fatty acids despite increased adipose tissue lipolysis.[228]

In PARADIGM-HF, at baseline 35% (2907/8274) of participants reported having diabetes mellitus.[229] Amongst those without a known diagnosis of diabetes mellitus, 39% (2103/5367) were found to have pre-diabetes (HbA1C between 6.0% and 6.5%) and 21% (1106/5367) had “undiagnosed diabetes mellitus” identified by a HbA1C of 6.5% or greater.[229]

In a post-hoc analysis of 3778 PARADIGM-HF participants (of which 1904 were allocated sacubitril/valsartan and 1874 allocated enalapril) who at screening had a diagnosis of diabetes mellitus or HbA1C of 6.5% or greater (98% of whom had type 2 diabetes mellitus), at 3 years post-randomization, sacubitril/valsartan was associated with a reduction in HbA1C of 0.14% (95% CI -0.23 to -0.06; P=0.0055), compared with

enalapril.[230] The reduction in HbA1C was only present in individuals reporting presence of diabetes at screening and did not correlate with baseline HbA1C level.[230]

Participants not on insulin therapy at baseline assigned to sacubitril/valsartan, compared with enalapril, had a 29% (114/1904 [7%] versus 153/1874 [10%] respectively; HR 0.71 [95% CI 0.56-0.90; P=0.0052) reduction in subsequent initiation of insulin therapy and, 23% (HR 0.77; 95% CI 0.58-1.02; P=0.073) lower risk of initiating oral hypoglycaemic treatments.[230] In patients without diabetes at screening, sacubitril/valsartan had no effect on initiation rates of insulin or oral hypoglycaemic agents.[230]

When risk of the primary outcome (hospitalisation for heart failure or cardiovascular mortality) was stratified by eGFR categories, declining eGFR in individuals with diabetes was associated with increased risk compared to individuals with normal kidney function and no diabetes.[229] An eGFR of 30 to 40mL/min in participants with diabetes was associated with about a 3-fold increase in risk of the primary outcome (HR 2.87; 95% CI 2.04-4.06) compared with individuals with normoglycemia. In people with diabetes even an eGFR of 90 mL/min or greater was associated with a 25% (HR 1.25; 95% CI 0.88-1.76) increase in risk of the primary outcome, compared with normal kidney function and no diabetes.[229] The beneficial effects of sacubitril/valsartan on reduction in risk of the primary outcome were unrelated to HbA1C level or diabetes status.[229]

High-density lipoprotein (HDL) cholesterol concentrations were marginally higher in those allocated sacubitril/valsartan, compared with enalapril (overall increase 0.02 mmol/L [95% CI 0.00-0.03]; P=0.043).[230] Both treatments produced a similar reduction in triglyceride levels (overall difference 0.01 mmol/L [95% CI -0.09 to 0.07]; P=0.83).[230]

Body mass index (BMI) increased by 0.28 (95% CI 0.14-0.41; P<0.0001) kg/m^2 in people assigned sacubitril/valsartan, compared with enalapril, despite favourable effects on other metabolic parameters.[230]

4.7 Sacubitril/valsartan in CKD and thesis aims

The data from the hypertension and heart failure trials of neprilysin inhibition with ARNIs suggests that this treatment may improve cardiovascular and renal outcomes in patients with CKD. However, the safety and efficacy of ARNI on renal progression and

albuminuria in patients with advanced CKD particularly those with significant levels of albuminuria are unknown.

The key aim of this thesis was to examine the effects of the ARNI sacubitril/valsartan in patients with CKD. All prior evidence of the effects of ARNIs in CKD had been extrapolated from animal models of CKD and from randomized trials of patients with mildly impaired renal function participating in trials of heart failure and hypertension. To fill this gap in evidence, a double-blind randomized controlled trial was undertaken to reliably test the effects of sacubitril/valsartan in people with CKD.

The United Kingdom (UK) Heart and Renal Protection (HARP)-III trial compared sacubitril/valsartan against irbesartan in 414 patients with CKD stages 3 and 4. The trial assessed the short-term safety and efficacy of sacubitril/valsartan in this patient population and was the first dedicated test of an ARNI in CKD.

The primary outcome of UK HARP-III was to compare the effect of sacubitril/valsartan with irbesartan (an ARB) on change in measured glomerular filtration rate (mGFR) from baseline to twelve months. Secondary aims of the trial examined in this thesis include the assessment of the effects of sacubitril/valsartan on urine albumin:creatinine ratio and eGFR. Tertiary aims assessed the short-term safety and tolerability of sacubitril/valsartan in people with CKD, its effects on blood pressure, rate of change in eGFR and biomarkers of cardiac damage (troponin I and NT-proBNP).

5 Methods

This Chapter describes the design, outcomes and statistical methods of the UK Heart and Renal Protection (HARP)-III trial.

5.1 UK HARP-III: a randomized-controlled trial

The UK HARP-III trial was a randomized, double-blind, placebo-controlled, multicentre trial comparing NEPi with sacubitril/valsartan with an ARB, irbesartan, in people with CKD stages 3 and 4.

Randomized controlled trials (if appropriately sized) provide the most reliable estimates of treatment efficacy.[231-233] Most treatments have only moderate effects on outcomes and proper randomization allows control of bias, enabling such moderate differences in outcomes to be assessed reliably.[131,233-237] Randomization ensures that each different type of patient entered into the trial will be allocated to each treatment in similar proportions, so any confounders (known or unknown) will also be equally distributed between the randomized groups.[234,235,237] Therefore, only random differences should affect the final comparison of treatment effects.[234,235]

Effective treatment allocation concealment (as well as proper randomization) is essential to prevent imbalances in prognostic factors and biased results.[238] The biases that could arise with inadequate allocation concealment could be of a similar magnitude to the treatment effect and therefore, the observed size of the treatment effect could appear much larger or smaller than it actually is or even be completely masked.[239-241]

Non-randomized treatments comparisons do not guarantee that any systematic differences between patients given each treatment were not just due to chance alone. For example, in such studies, foreknowledge of the treatment allocation would result in selection bias (i.e. only those participants deemed to have the greatest benefit from the treatment would be entered in the trial) and biased reporting of outcomes of interest.[232,242] As a result, such comparisons could produce the wrong result since the potential biases and random error could erroneously show the treatment as being beneficial, harmful or having no effect due to differences in outcomes that are unrelated to the treatment being tested.[131,234,235,242]

5.2 Trial aims

The UK HARP-III trial aimed to randomize at least 400 participants aged 18 years or over with CKD (eGFR of 20 or above and below 60 mL/min/1.73m^2) to receive either sacubitril/valsartan or irbesartan and, to follow them up for 12 months. The trial protocol was amended in May 2015 to extend follow-up from the originally planned 6 months to 12 months in light of new data that suggested sacubitril/valsartan took longer (about 9 months) to have full effect.[200] Irbesartan was chosen as the comparator as it has a license for treatment of proteinuric CKD.[243]

The primary aim of UK HARP-III was to assess the difference in effect of sacubitril/valsartan, compared with irbesartan, on mGFR (measured using ^{51}Cromium-ethylenediaminetetraacetic acid [^{51}Cr-EDTA], or ^{99}mTc-diethylenetriaminepentaacetic acid [DTPA], or iohexol methods depending on local practice) at 12 months. Secondary aims included effects of sacubitril/valsartan on albuminuria and eGFR in people with CKD (Table 6). Tertiary aims included assessment of safety and tolerability of sacubitril/valsartan and its effects on blood pressure, rate of change in eGFR and biomarkers of cardiac damage (troponin I and NT-proBNP) (Table 6).

Primary

mGFR at 12 months

Secondary

- Study average uACR
- Study average eGFR

Tertiary

- Study average systolic and diastolic BP (mmHg)
- Rate of change of eGFR calculated from creatinine values at Randomization, 1, 3, 6, 9 and 12 months (overall, and separately for 0-3 months [i.e., Randomization, 1 and 3 month values] and 3-12 months [i.e., 3, 6, 9 and 12 month values]) using the CKD-Epidemiology Collaboration formula. Where values from the central laboratory are available (randomization, 3, 6 and 12 months) these will be used, but local values will be used at 1 and 9 months.
- Cardiac biomarkers (troponin I and NT-proBNP) at 6 and 12 months

Table 6: UK HARP-III trial aims

BP = blood pressure; CKD = chronic kidney disease; eGFR= estimated glomerular filtration rate; mGFR = measured glomerular filtration rate; NT-proBNP = N-terminal of the prohormone brain natriuretic peptide; uACR = urine albumin:creatinine ratio

5.3 UK HARP-III trial population

Men and women aged 18 years or over with CKD (based on eGFR results for at least three months estimated using either the modification of diet in renal disease [MDRD] or CKD-Epidemiology Collaboration [CKD-EPI] formulae) were eligible to participate if they had:

- an eGFR of 20 or greater but less than 45 mL/min/1.73m^2, OR
- an eGFR of 45 or greater but less than 60 mL/min/1.73m^2 AND a uACR greater than 20 mg/mmol (or urine protein:creatinine ratio [uPCR] greater than 30 mg/mmol)

The original trial protocol required all patients to have a uACR greater than 20 mg/mmol irrespective of eGFR. This was subsequently changed by the trial steering committee in February 2015 to apply to only those individuals with an eGFR of 45 mL/min/1.73m^2 or greater but less than 60 mL/min/1.73m^2 to aid recruitment. In addition to satisfying all of the eligibility criteria, patients were only considered as potentially eligible to participate if their nephrologist did not believe there was any definite indication for or contraindication to sacubitril/valsartan and none of the exclusion criteria were met (Table 7).

The inclusion criteria were kept deliberately broad and simple to enable inclusion of a wide range of patients with CKD (in whom the treatment effects are uncertain), ensuring that the trial results would be widely generalizable and could illustrate the overall treatment effect more reliably in this population (in addition to making recruitment easier and more efficient).[235,237]

Inclusion criteria

- Men or women aged ≥18 years (at screening)
- Established CKD:
  - eGFR ≥20 <45 mL/min/1.73m^2 (estimated with the MDRD formula); or
  - eGFR ≥45 <60 mL/min/1.73m^2 and uACR >20 mg/mmol

Exclusion criteria

- ARB therapy contraindicated e.g. bilateral renal artery stenosis
- Known intolerance of ARB
- Current treatment with aliskiren (a direct renin inhibitor)
- Mean systolic blood pressure >180 mmHg at screening visit (or investigator unwilling to withdraw ACEi or ARB for another reason)
- Serum potassium >5.5 mmol/L
- Patients that currently have nephrotic syndrome (i.e. uPCR >350 mg/mmol [or uACR >300 mg/mmol] AND serum albumin <30 g/L) or if patients are currently receiving immunosuppression to treat the nephrotic syndrome
- Functioning renal transplant
- Acute coronary syndrome, stroke or transient ischaemic attack in 3 months prior to screening
- Known chronic liver disease or alanine aminotransferase (ALT) / aspartate aminotransferase (AST) >2x upper limit of normal (ULN) at screening
- History of angioedema (drug-related or otherwise)
- Use of unlicensed investigational medicinal product in previous month
- Pregnancy, lactating women, or women with child-bearing potential (refusing a reliable method of contraception)
- Medical history that might limit the patient's ability to take study treatments for the duration of the study (e.g. severe respiratory disease, or recent history of alcohol or substance misuse or history of cancer or evidence of spread in last 5 years other than non-melanoma skin cancer)

Table 7: UK HARP-III trial inclusion and exclusion criteria
ACEi = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; eGFR = estimated glomerular filtration rate; uACR = urine albumin:creatinine ratio; uPCR = urine protein:creatinine ratio

5.4 Trial design

The trial design and detailed information regarding the study schedule are summarised in Figure 7.

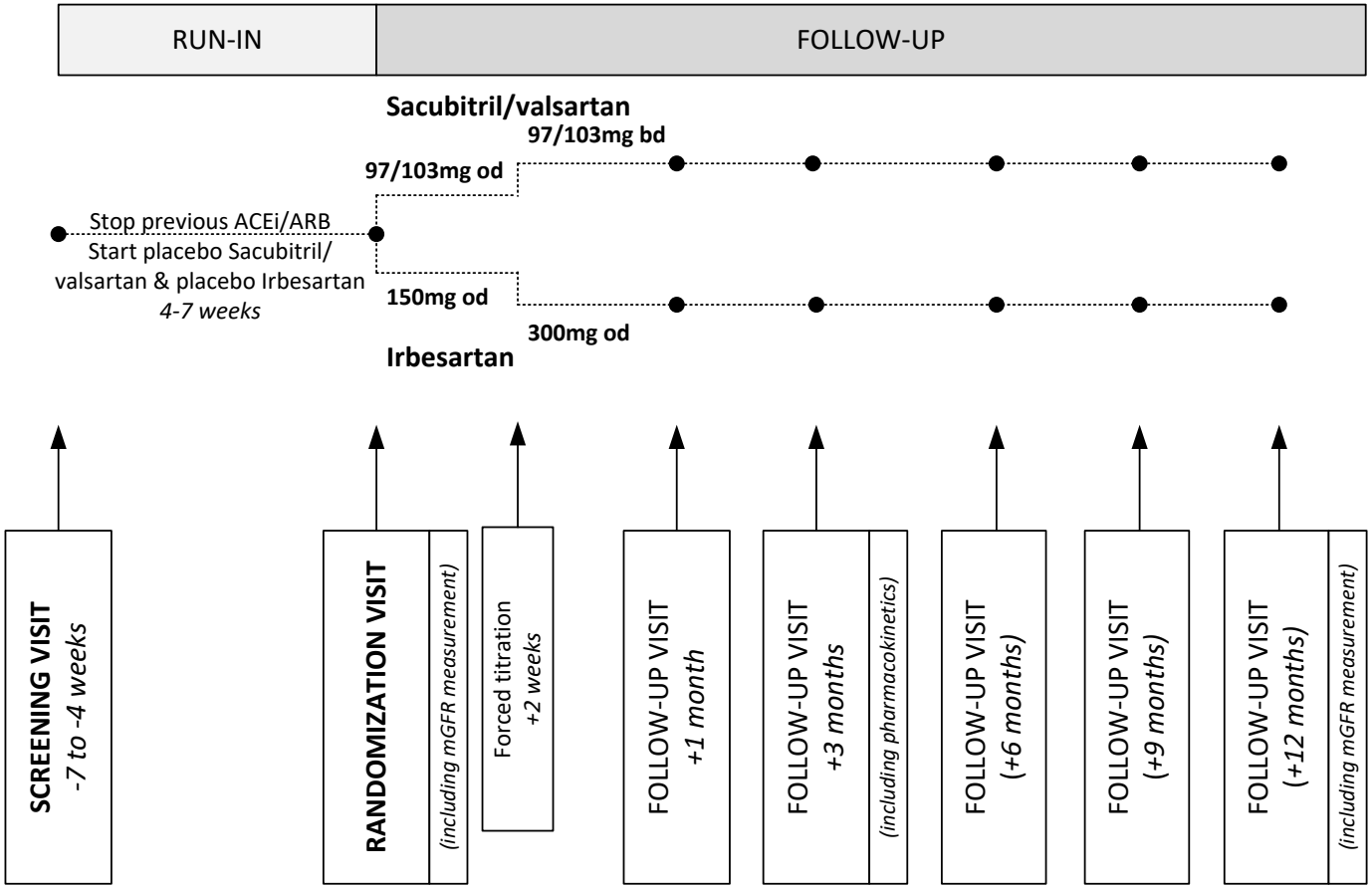


Figure 7: UK HARP-III trial design overview
ACEi = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; mGFR = measured glomerular filtration rate

5.4.1 Central coordination of the trial

5.4.1.1 *Trial organisation*

The University of Oxford was the trial sponsor. The Central Coordinating Office (CCO) for UK HARP-III was based at the Clinical Trial Service Unit & Epidemiological Studies Unit (CTSU), University of Oxford. At each local study site, a senior nephrologist was designated as the local lead investigator (LLI) and local research nurses (local research coordinator [LRC]) worked together with the LLI to identify, recruit and follow-up study participants.

5.4.1.2 *Staff training*

Prior to initiating recruitment, LRCs received training in the study procedures and the web-based data capture system at the CCO. The web-based system enabled direct entry and recording of data to avoid any data transcription errors. Research staff conducting trial visits and procedures undertook a series of exercises following the training to confirm that they understood the study protocol and procedures and were confident with using the web-based study information technology (IT) system. Additional training was provided if indicated.

The LLIs and LRCs were provided with all materials relevant to the study procedures to enable them to perform the daily study-related tasks, including a Manual of Operations.

During the recruitment phase, regular teleconferences were arranged for the research nurses to provide additional advice and support and to answer any general/non-urgent queries. In addition, annual meetings were held for the research nurses to be updated on study procedures and progress.

5.4.1.3 *Data management*

The UK HARP-III study IT system, *Cello*, was a web-based direct data entry system. CCO staff used the system to manage drug supply, track adverse events, and for central monitoring of the study. All access required a unique username and password, and any changes to data required the user to enter their username and password as an electronic signature. Staff access was restricted according to their role in the study.

5.4.1.4 *Trial treatments*

The manufacturer of sacubitril/valsartan (Novartis) provided both study treatments and their matching placebos. Cases of study treatment were shipped at appropriate

intervals to the local centres (guided by remaining supplies at local sites) at the request of administrators at the CCO. Study treatment packs consisted of two bottles: one containing active or placebo tablets of sacubitril/valsartan and the other containing active or placebo capsules of irbesartan.

Study treatments were stored at or below 25°C (with regular temperature monitoring) and LRCs were trained on how to issue study treatments as guided by *Cello*. Issue of study treatments required the entry of a verification code from the study treatment pack to ensure that the correct treatment pack was selected for the participant, at sites where LRCs issued study treatment themselves. At those sites where LRCs were not permitted to issue study treatment, it was stored and issued from the local site pharmacy, but the verification code check was not available. Local pharmacy staff were responsible for ensuring that the correct pack was issued to the participant by manually checking the verification code against the issuing information provided by *Cello*.

5.4.2 Local clinical centres

At each of the local study sites, a local clinical centre (LCC) was established. LCC staff were responsible for assisting with obtaining local research approval, conducting the trial procedures according to the study protocol, dealing with routine enquiries from patients and their families or other personnel and obtaining and providing the relevant information for study outcomes (e.g. laboratory and mGFR results).

The LLI was responsible for trial oversight at the LCC and ensuring that the study protocol was adhered to. The LLI could delegate duties (specified on the delegation of duties log) to other staff members (e.g. permitting study nurses to take informed consent and perform all trial visits) but retained overall responsibility for all aspects of the study conducted at the LCC. LCCs were established at 24 sites (Appendix 2: Supplementary material) across the UK.

I travelled to several sites to address any issues local staff had with establishing the LCC (for example, concerns relating to issuing study treatment). The visits increased awareness of the trial amongst clinical staff and encouraged participant recruitment through the use of the pre-screening method.

5.4.3 Identification and invitation

UK HARP-III piloted a new method of recruiting participants into renal trials to test whether such methods enabled faster and more efficient recruitment.

Local site study staff were advised to identify potentially eligible patients primarily from electronic hospital databases. Study staff could supplement this with identification of patients from lists of clinic attendees, consultant referrals and patients self-referring (for example, from having viewed the study poster [Appendix 3: study poster] or visiting the study website). Once identified, a brief check of suitability for participation was performed using hospital medical records for any obvious reason for ineligibility before patients were invited.

Potentially eligible patients were then sent a copy of the invitation letter and Participant Information Leaflet (PIL; Appendix 4) or provided with this in clinic on behalf of the LLI. Study staff then telephoned these individuals about a week later to discuss the trial with them in detail, answer any questions or concerns they might have and to check whether they were interested in participating.

Interested individuals were offered an appointment to attend a screening visit and some basic demographic information was entered into the study IT system after obtaining verbal consent.

5.4.4 Screening visit

At the screening visit, eligibility was checked by asking questions pertaining to the inclusion and exclusion criteria (Table 7) and answers were recorded directly into the screening case report form in the study database. If any of the exclusion criteria were met and patients were deemed ineligible the visit ended at this point. For eligible patients, after written informed consent was obtained, information regarding relevant medical history (including primary renal diagnosis, cardiovascular disease, and presence of diabetes mellitus) and current medications (including total daily doses of all anti-hypertensive medications) was recorded. Blood pressure was measured three times after sitting for at least 5 minutes (using an Omron M6 automated digital sphygmomanometer) in addition to height and weight, and these measurements were recorded in the visit form.

5.4.4.1 *Consent*

Patients who appeared eligible to participate had the study explained to them by the LRC using the Participant Information Leaflet (Appendix 3) as a basis for discussion.

Patients were offered the opportunity to discuss the trial further with their family or friends, General Practitioner or usual nephrologist, and those patients who wished to do so were asked to attend at a later date for a repeat screening visit. Patients who were unlikely to be willing or able to continue taking study treatments and, comply with attendance at study follow-up visits for entire the duration of the trial were discouraged from participating. Consent was also sought for the storage of blood and urine samples.

5.4.4.2 *Biological samples*

Blood and urine samples were collected at the screening visit and sent to the local laboratory for analysis to confirm eligibility for the trial. Participants provided a random urine sample in clinic for quantification of albuminuria and were given a container to collect a first morning void urine sample on the day of the randomization visit. 24 hour urine collections are the gold standard method for measuring urine albumin excretion and verifying the presence of microalbuminuria.[244] However, measurement of albuminuria in first morning void urine samples have been shown to have high agreement with albuminuria quantified from a 24 hour collection, much less intraindividual variability and, are much more practical.[244] Random spot urine collections are associated with significant variation and are therefore not recommended.[244]

If the results of biological samples collected at screening were deemed to be inaccurate, and therefore did not meet the eligibility criteria, the result could be repeated once, and the latest result was used to confirm eligibility. If the eGFR result was 60 or greater but less than 70 ml/min/1.73^2 on the sample sent at screening, such patients were eligible to continue in run-in if all other eligibility criteria were met.

5.4.5 Pre-randomization run-in

Following the screening visit, any current ACEi and/or ARB therapy that the patient was taking was stopped and the participant entered the single-blind placebo pre-randomization run-in period which lasted between four and seven weeks prior to randomization. Participants were issued with a run-in treatment pack containing an eight-week supply of study treatment and advised to take one tablet of placebo sacubitril/valsartan and one capsule of placebo irbesartan daily. Participants were also provided with a study treatment information sheet (STIL; Appendix 5).

The results of blood and urine samples along with the clinical data collected at screening were reviewed by the LLI (or another nephrologist approved by the LLI) to approve the participant as suitable for randomization, if appropriate. Those participants not eligible for the trial on the basis of results of samples taken at screening, or for any other reason in the opinion of the LLI, were telephoned by research staff daily and informed. They were advised to stop the study run-in treatment and restart any ACEi and/or ARB treatment that was withdrawn at screening.

If raised BP was a concern during run-in, then the LLI or the participants' usual nephrologist was advised to titrate up existing antihypertensive medications (if possible) or to start additional treatment but to avoid ACEi, ARB or DRI. The choice of additional antihypertensive medication remained at the discretion of the local nephrologist.

Participants could withdraw from run-in at any time prior to randomization. Participants who did not withdraw returned between four and seven weeks later to have their GFR measured (using ^{51}Cr-EDTA, ^{9}mTc-DTPA or iohexol) and attend for the randomization visit. The results of the mGFR test were not required to determine eligibility for randomization, as this was based on the eGFR at screening. Copies of the mGFR result were sent back to the CCO for confirmation of the result by CCO clinical study staff blind to treatment allocation.

In willing participants, a voluntary 24-hour urine collection was undertaken 24-48 hours prior to the randomization visit for quantification of albumin and sodium excretion (but this was not required for determining eligibility for randomization).

The aims of the pre-randomization run-in were: (i) to allow a 'wash-out' of any ACEi prior to potential treatment with NEPi (in light of the risk of angioedema when the two treatments are combined); (ii) to allow a comparison of the acute effects of study treatments on GFR; and (iii) to improve compliance by excluding people less likely to adhere with study treatment and thereby reduce the rate of post-randomization discontinuation of study treatments and consequently improve the trial's statistical sensitivity.[245-248]

5.4.6 Randomization

At the randomization visit, participant's eligibility and willingness to participate was re-confirmed. Participants were asked about any serious adverse events since their screening visit and compliance with study treatment was checked. Study treatments

were required to be taken on average at least six days per week (over 80% of the time) for compliance to be regarded as satisfactory. Pill counts were not performed at any study visits as these are unreliable and tend to overestimate adherence with treatment.[233,249-251] Non-study medications were also checked and updated. Blood pressure and weight were measured and recorded.

Participants were not eligible for randomization if: systolic blood pressure was less than 110 mmHg (or less than 130 mmHg if they had symptoms of hypotension); or if the LLI was concerned about hypotension); or if they reported an adverse event that they believed to be related to their (placebo) run-in treatment. The protocol was amended in February 2015 (Section 6.2.1.1) to allow inclusion with this lower cut-off (110 mmHg) for blood pressure at randomization, with no upper limit (previously set as systolic blood pressure of 180 mmHg) as blood pressure would be controlled with active study treatment following randomization.

Participants who: remained willing and eligible; tolerated the run-in medications; were willing to comply with taking study treatments; and attend follow-up visits for the trial duration (a further 12 months) were eligible to be randomized.

Participants were randomized 1:1 by *Cello* which prevented any foreknowledge of treatment allocation affecting the decision to enter a patient into the trial (i.e. selection bias which would result in patients being systemically different between the treatment two arms).[234,235,238] Random allocation of treatment ensured that each participant that entered into the trial had a similar chance to being allocated to either treatment and in similar numbers in each group.[235]

Effective treatment allocation concealment (as well as proper randomization) is essential to prevent imbalances in prognostic factors and biased results.[238] The biases that could arise with inadequate allocation concealment could be of a similar magnitude to the treatment effect and therefore, the observed size of the treatment effect could appear much larger or smaller than it actually is or even completely masked.[239-241]

A minimization algorithm was used to ensure that the treatment groups were balanced with respect to prognostically important factors that could impact the treatment affect in patients with CKD,[252,253] particularly predictors of renal progression (measured at screening) including: age, sex, systolic blood pressure, eGFR, uACR and presence of diabetes mellitus.[252-254]

5.4.6.1 *Randomized treatment and blinding*

At the end of the randomization visit, run-in treatment was collected, and participants were issued with a treatment pack containing two bottles of study treatments: one containing tablets of sacubitril/valsartan 97/103 mg or placebo and the other containing capsules of irbesartan 150 mg or placebo. As both treatments differed in their appearance (i.e. tablets and capsules) a "double-dummy" approach was used to ensure both participants and all study staff remained blind to treatment allocation to minimize bias (such as ascertainment, recall and detection bias).[131] The two treatment arms that participants were randomized to were as follows:

- Sacubitril/valsartan plus placebo irbesartan

OR

- Irbesartan plus placebo sacubitril/valsartan

Participants were initially advised to take one tablet and one capsule daily (i.e. either sacubitril/valsartan 97/103 mg and placebo irbesartan or placebo sacubitril/valsartan and irbesartan 150 mg) for the first two weeks post-randomization.

The participant's usual nephrologist and GP were then informed that the participant had been randomized into the UK HARP-III trial and advised to avoid any ACEi, ARB or DRI for the duration of the trial (if possible). If their managing doctor felt that the participant should be started on any ACEi, ARB or DRI treatment during the course of the trial, they were advised to discuss this first with the LLI or with a CCO clinician, following which the participant could stop their allocated study treatment but would continue to attend all remaining follow-up visits. Managing nephrologists were asked not to alter antihypertensive therapy during the first four weeks after randomization unless absolutely necessary on clinical grounds.

5.4.6.2 *Blood and urine samples*

Blood and urine samples were collected for local analysis of creatinine, electrolytes (sodium and potassium), liver function tests (alanine aminotransferase [ALT] or aspartate aminotransferase [AST] and bilirubin) and uACR. Participants also had additional venous blood collected into an EDTA-containing polyethylene tube which was kept refrigerated until processing on the same day. The sample was then centrifuged at 1500g and the plasma aliquoted into three 2 mL cryovials, and additionally two 2 mL cryovials were filled with urine. These samples were then frozen and stored at or below -20°C locally, prior to transfer for analysis (at the end of the study) at the CCO's Wolfson laboratory in Oxford (Table 8).

Analyte	Time point			
	Randomization	3 months	6 months	12 months
EDTA plasma samples				
Creatinine	X	x	x	x
Albumin	X		x	x
Troponin-I	X		x	x
NT-proBNP	X		x	x
Urine samples				
uACR	X	x	x	x

Table 8: Planned central laboratory blood and urine analyses
NT-proBNP = N-terminal of the prohormone brain natriuretic peptide; urine albumin:creatinine ratio

5.4.7 Forced titration at 2 weeks post-randomization

At 2 weeks post-randomization, participants were asked to attend either their local study centre or general practice to have a blood sample taken to check their renal function and potassium. If the results were satisfactory, participants were instructed to increase the dose of study treatments to the maximum dose, taking one tablet twice daily and two capsules once daily (i.e. either sacubitril/valsartan 97/103 mg twice daily and placebo irbesartan once daily or placebo sacubitril/valsartan twice daily and irbesartan 300 mg once daily). If based on these results or due to some other concern (such as hypotension) the LLI believed it was inappropriate to titrate the study medications, the participant was advised to remain on the lower dose of study treatments.

5.4.8 Follow-up visits (1, 3, 6, 9 and 12 months)

Study follow-up visits were scheduled at 1, 3, 6, 9 and 12 months post-randomization, at which LRCs systematically sought information on adverse events, symptoms of hepatitis and whether the participant had required dialysis since their previous study visit. The visits could be undertaken up to two weeks either side of the scheduled visit date.

5.4.8.1 *Adverse events and compliance with trial treatment*

At each study visit, details of all serious adverse events and non-serious adverse events that the participant believed to be related to study treatment or leading to its discontinuation were recorded directly into the study IT system. Any serious adverse events that were considered to be due to study treatment, were discussed immediately with a CCO clinician, as such events may have required expedited

reporting. An estimation of adherence with study treatment was made from direct questioning of participants and pill counts were not performed.[233,249-251]

5.4.8.2 *Physical measurements*

The participants weight and blood pressure were measured at every study visit. Blood pressure was measured and recorded three times after the participant had been seated for at least five minutes (using an Omron M6 automated digital sphygmomanometer). Blood pressure was controlled according to the current Kidney Disease Improving Global Outcomes (KDIGO) guidelines for managing blood pressure in patients with CKD.[59] Blood pressure measurements recorded on visit forms were monitored by CCO clinicians and if there was a concern, LRCs were contacted and advised to discuss this with their LLI or the participants usual nephrologist. The choice of any additional (or withdrawal of) anti-hypertensive treatment remained at the discretion of the responsible clinician if required.

5.4.8.3 *Blood and urine samples*

At every follow-up visit samples were taken for local measurement of creatinine, electrolytes (sodium and potassium), liver function tests (ALT or ALP and bilirubin) and uACR. Results of these samples were to be entered into the study IT system within 2 working days and were reviewed daily by a CCO clinician, who provided advice on any abnormal results including any potassium more than 5.5 mmol/L, ALT more than twice the upper limit of normal or a fall in eGFR more than 25% from the previous value.

At the specified visits (3, 6 and 12 months), participants also had an additional blood sample collected in an EDTA-containing tube which was centrifuged and the plasma aliquoted into three cryovials, with two cryovials filled with urine and these samples were then frozen at or below -20°C locally, prior to transfer for analysis in the Wolfson laboratory (Table 8).

Participants were provided with a container at the previous visit (randomization, 1, 3, 6 and 9 months) and asked to bring a first morning void urine sample with them to every clinic visit. If they did not bring a first morning void sample, a random sample was provided by the participant when they attended the study clinic. The samples were then transported to the coordinating centre's Wolfson laboratory for analysis and long-term storage.

5.4.8.4 *Central laboratory methods*

Creatinine was assayed in the central laboratory on a Beckman Coulter AU680 analyser using a kinetic alkaline picrate method and calibrated using material traceable to isotope dilution mass spectrometry (using the National Institute of Standards and Technology Standard Reference Material 967); troponin I was measured by immunoassay on an Architect system and NT-proBNP by immunoassay on an Elecsys system.[255] All analyses were undertaken at the coordinating centres' Wolfson Laboratory, University of Oxford.

5.4.8.5 *Issuing trial treatment*

Participants were reminded at every study visit regarding the importance of avoiding the use of any non-study ACEi, ARB or DRI and, asked to inform local study staff or to contact the CCO if any such treatment was prescribed. At the 3, 6 and 9 month visits, participants were re-issued a further supply of their randomized study treatment (as each treatment bottle contained 105 days' supply). Participants were asked to return their previously allocated supplies back to the LRC.

5.4.8.6 *Final follow-up and mGFR*

All participants were requested to have a second GFR measured using ^{51}Cr-EDTA, ^{9}mTc-DTPA or iohexol (with the same method being used for all measurements in trial participants at that centre) depending on local practice, even if they had previously stopped study treatment. In most cases this was performed on the same day as their final follow-up visit but if this were not possible, it was arranged during the two-week period prior to their scheduled final follow-up visit. Copies of the mGFR test result were sent back to the coordinating centre for confirmation of the result by clinical trial staff blind to treatment allocation to ensure an unbiased assessment of the primary outcome data.

Once participants had completed the final visit, a letter was sent to the usual nephrologist and GP (and a copy provided to the participant in clinic) informing them of this and with advice on restarting any prior ACEi and/or ARB 48 hours after the last dose of study treatment (to prevent any risk of angioedema with overlap of NEPi and ACEi). Participants and study staff were also asked to report any serious adverse events that occurred within 15 days of the final trial visit.

5.4.9 Early recall visits

At any point following randomization prior to the final follow-up visit, an 'early recall visit' (i.e. additional trial visits outside of the scheduled timeline) could be arranged. This could be done at the request of the participant (e.g. if they are experiencing troublesome symptoms) or on the advice of CCO or local trial staff (e.g. on the basis of the local blood results taken at a preceding trial visit). Abnormal potassium, creatinine, or ALT/AST results in samples taken from a trial visit prompted an early recall to enable repeat checking.

5.4.10 *Modifying or discontinuing trial treatment*

Participants were encouraged to adhere to trial treatments throughout the study. However, the LLI could decide to discontinue trial treatment at any time in the interests of the participant's health and well-being. For example, trial treatment may be temporarily or permanently discontinued for a particular participant if one of the following criteria were met:

- serious adverse events thought likely to be due to trial treatment (suspected serious adverse reaction [SSAR]). This usually resulted in permanent discontinuation of study treatments unless the investigator and CCO agreed that there was justification to continue or restart the study treatment
- participant started a contraindicated medication (i.e. ACEi, ARB or DRI) for the duration of use of the contraindicated medication
- conditions or procedures in which study treatments may be contraindicated (e.g. diagnosis of severe bilateral renal artery stenosis)
- pregnancy
- any other situation where, in the opinion of the participant's own doctors or the clinic staff, continuing trial treatments would not be in the participant's best interest

In addition, the participant themselves may have decided not to continue trial treatments. If trial treatment was discontinued, the participant was still followed-up for the duration of the trial in the usual way (wherever possible). Complete follow-up of such data was essential as all planned analysis employed the 'intention-to-treat' (ITT) principle.[236,256] Participants who could not tolerate the full dose of their allocated treatment (i.e. sacubitril/valsartan 97/103 mg twice daily or irbesartan 300 mg once daily) could halve their dose. If appropriate, participants were encouraged to increase their dose again once any symptoms had resolved.

5.4.11 *Withdrawal from the trial*

Participants had the right to withdraw from the trial at any time and for any reason (without prejudice to his or her future medical care by the physician or at the institution where they are usually cared for) and were not obliged to give reasons for doing so. If participants were no longer willing to attend trial clinics, then they were asked if they would be willing to continue follow-up by telephone and/or relevant data (e.g. eGFR) collected directly from their medical records, unless participants withdrew consent for this as well. If participants withdrew their consent from all forms of follow-up, they were asked to confirm this in writing and the local investigator was asked to complete a "Withdrawal of Consent" form to confirm this.

5.5 Statistical analysis

A summary of the main details of the statistical analysis plan are presented below. Further details are provided in the published Data Analysis Plan (Appendix 1: Supplementary material).

5.5.1 Sample size calculation

The primary aim of the trial was to compare the change in mGFR between sacubitril/valsartan and irbesartan from the baseline value to final follow-up. This could be done most efficiently through use of an analysis of covariance (ANCOVA) which compared mean follow-up mGFR between the two treatment groups after adjustment for baseline mGFR (and of any random imbalances in baseline measurements that occurred between the treatment groups despite randomization).[233,257-259] ANCOVA has greater statistical power to detect treatment effects than other methods.[233,258]

Assuming a between person standard deviation in mGFR of 15 mL/min/1.73m^2 and a correlation between an individual's baseline and follow-up mGFR of 0.8, randomization of 400 participants would provide 80% power (at 2p=0.05) to detect a difference in mGFR at final follow-up of 3 mL/min/1.73m^2 (the chosen minimum clinically meaningful difference), even if 15% of participants discontinued study treatment after randomization.

5.5.2 Intention to treat analysis (ITT)

Analyses of all outcomes were performed using the ITT, i.e. all comparisons compared outcomes among all participants randomized to receive sacubitril/valsartan 97/103 mg twice daily versus all those randomized to receive irbesartan 300 mg once daily, regardless of whether or not the participant ever actually took the study treatment or the duration that they took it for.[234,236,256] ITT analyses avoids introducing systematic errors relating to differences between participants who deviated from one treatment compared with the other and, in doing so, provides a more reliable assessment of whether there is any true difference between the treatments being tested and their effects on the outcomes of interest (compared with for example 'on-treatment' analysis).[233,234,236,238]

5.5.3 Methods of analysis

5.5.3.1 *Analysis of Covariance (ANCOVA)*

Comparisons of continuous variables (listed in Table 9) between the allocated treatment arms were performed using ANCOVA adjusted for each patient's value at randomization.[258] If continuous variables were not normally distributed then appropriate transformations (for example log transformation) were made.[260,261]

Categorical variables	Continuous variables
Sex† Ethnicity† CKD stage* Prior cardiovascular disease† Prior diabetes mellitus† Non-trial medications* Renin-angiotensin system inhibitor use† Primary renal diagnosis†	Age† Systolic blood pressure* Diastolic blood pressure* Weight* Height† CKD Epidemiology Collaboration formula eGFR* Urine albumin:creatinine ratio* Potassium* Aspartate aminotransferase or Alanine aminotransferase* Albumin* 24-hour sodium excretion 24-hour albumin excretion Measured glomerular filtration rate N-terminal of the prohormone brain natriuretic peptide ‡ Troponin I‡

Table 9: Continuous and categorical data in UK HARP-III
*Repeated at every follow-up visit. †Recorded at screening. ‡Measured centrally on samples at the time-points stated in Table 8.

5.5.3.2 *Repeated measures analyses of biomarkers*

In cases where more than one value was available at follow-up of a biomarker (Table 8), a comparison of the mean value of the biomarker was performed at each follow-up time using ANCOVA adjusted for the baseline value of the biomarker (to reduce variance and bias).[233,237,262]

5.5.3.3 *Imputation of missing data*

As all analyses were performed according to the ITT principle, in cases where there was data missing for primary and secondary outcomes, data was imputed (unless the participant had died prior to the relevant time-point). For each of the continuous outcomes missing post-randomization results were imputed, using 20 imputed datasets (generation of 10 or more imputed datasets have been shown to provide 95% or greater efficiency without significant change in the precision of the results),[263,264] with results across the imputations combined using the method of Rubin.[263]

Multiple imputation generated a plausible range of values that approximated the missing value and took into account the variability in the results between the imputed datasets and the uncertainty with the missing results.[263-265] It therefore allowed all participants to be included in the analysis, even those with missing data.[264]

Multiple imputation is superior to other methods of handling missing data. Use of only "complete-cases" (i.e. observations with no missing data), would lead to substantially biased results since complete-cases may be very different to those who are not.[264-267] In addition, missing data across several variables would result in a substantial proportion of the sample population being excluded resulting in significant loss of trial power, precision in estimates and misleading conclusions.[264-266]

Single versus multiple imputation would also have been inferior as the imputed value is inserted and analysed as if it was the actual value observed.[266,267] However, single imputation underestimates the uncertainty in the imputed values and so the variance becomes progressively and inappropriately smaller and artificially increases the precision of the estimate and the likelihood of a type 1 error.[266]

Last observation carried forward is another single imputation technique that has been used in which the missing value is assumed, incorrectly, to have remained unchanged from that previously recorded and therefore, the last recorded value is used to replace the missing value.[266,267] This approach can also significantly underestimate the result and is therefore misleading and not generally used.[266,267]

The multiple imputation procedure used in UK HARP-III trial analyses took into consideration each participant's key baseline characteristics (Appendix 1: Supplementary data, section 3), treatment allocation and any intermediate follow-up values of the biomarker, where available.

5.5.3.4 *Imputation of the primary outcome data*

Participants with a missing randomization mGFR value had their eGFR value at randomization imputed in place of the missing result. Participants without a final mGFR, had the missing mGFR result imputed using multiple imputation. For any participants who progressed to ESKD and commenced chronic dialysis during the trial, a value of 0 was imputed for their 12-month mGFR.

Technical reasons can lead to spurious measured GFR results which would not be apparent until after the participant had been randomized into the trial and initiated on randomized treatment (or when they stopped taking randomized treatment at the end of the trial). The differences between each mGFR value and its corresponding creatinine-based eGFR value (collected at the same timepoint as the mGFR test) was calculated and the distribution of the differences were inspected prior to any unblinded analyses being performed. Where the difference between the mGFR result and the central eGFR was more extreme than the 1st or 99th centile of the distribution of differences, the value of mGFR was set to missing in those cases. Multiple imputation was used to calculate missing mGFR values. The results from the multiple imputation analyses were compared with those from equivalent "complete-case" analyses, but primary emphasis was placed on the results after multiple imputation.

5.5.4 Analysis of tertiary outcome: rate of change in eGFR

The rate of decline in eGFR in those randomized to sacubitril/valsartan and irbesartan was assessed using creatinine measurements analysed on central samples collected at randomization, 3, 6 and 12 months and using local creatinine results collected at 1 and 9 months.

Missing values for eGFR were imputed using multiple imputation and participants with the poorest fitting slopes (defined as participants with the mean deviation from their own fitted slope in the top 1% of the distribution [of mean deviations across all participants]) were excluded.

5.5.5 Allowance for multiplicity of comparisons

The primary outcome was assessed without adjustment for multiplicity. For secondary and particularly the tertiary and exploratory analyses, allowance in their interpretation was made for multiple hypothesis testing,[236,256] taking into account the nature of events (including timing, duration and severity) and evidence from other studies. In addition to the pre-specified comparisons, many other analyses were performed with due allowance for their exploratory and, perhaps, data-dependent nature.[268]

Conventionally, two-sided P-values less than 0.05 are often described as “significant”. But, the larger the number of events on which a comparison is based and the more extreme the P-value (or, analogously, the further the confidence interval is from zero) after any allowance has been made for the nature of the particular comparison (i.e. primary, secondary or tertiary; pre-specified or exploratory), the more reliable the comparison and, hence, the more definite any finding will be considered.[268]

5.5.6 Tests for heterogeneity

The sample size was powered to detect the overall difference in treatment effect between the two groups and therefore the numbers of patients within any subgroups would be inadequate to produce a result that could reliably estimate the treatment effect in any such subgroup (which would require a substantially larger sample size).[238,268,269] When a number of different subgroups are considered, chance alone may lead to there being an apparent lack of effect (or even a magnified or weakened effect) in several subgroups in which the treatment effect is really only about the same as that observed overall.[268-270] In such circumstances, “lack of direct evidence of benefit” is not “good evidence of lack of benefit”, and any obvious significant overall result would provide substantial indirect evidence of benefit in some small subgroups where the actual results, if they were taken in isolation, were not conventionally significant (or could even be harmful).[236,256,270] Therefore, unless the proportional effect in some specific subgroup is clearly very different from the overall observed result, the true treatment effect in that subgroup is best estimated indirectly by applying the proportional effect observed among all patients in the trial to the absolute risk of the event observed among control patients in that category.[268,270]

To account for this, tests for heterogeneity of the proportional effect observed in subgroups were used (with allowance for multiple comparisons) to determine whether the proportional effects in specific subgroups were clearly different from the overall estimated treatment effect.[236,256,268] If, however, three or more patient categories could

be arranged in some meaningful order (e.g. age at randomization: less than 60; 60 or greater but less than 70; 70 or above) then assessment of any trend was made. For subgroups based on continuous variables (Table 9), approximate similar sized divisions (such as by tertiles) were used, using natural cut-offs to define categories (e.g. systolic blood pressure less than 140 mmHg as opposed to less than 138.7 mmHg). These cut-offs were defined exactly prior to any unblinding of results.

5.6 Trial governance and approvals

The Ethics Committee meeting at which the UK HARP-III trial was reviewed took place at the Nottingham Research Ethics Committee (REC)-2 panel on 25th November 2013. I attended the meeting with the Chief Investigator. REC approval (13/EM/0434) was granted on 17th December 2013. UK HARP-III acquired Medicines and Healthcare Products Regulatory Agency (MHRA) approval (Clinical Trial Authorisation [CTA] 21439/0243/001-0001) and, obtained local NHS research and development (R&D) committee and Administration of Radioactive Substances Advisory Committee (ARSAC) approvals at all 24 sites across the UK prior to initiation of the trial. The trial manager coordinated the submission of all approvals via the centralised Integrated Research Application System (IRAS).

UK HARP-III was registered with the International Standard Randomized Controlled Trial Number (ISRCTN) registry (ISRCTN11958993) prior to recruitment of the first participant. All clinical trials require registration with ISRCTN so that there is transparency of the whole trial, from development of the protocol through to publication of trial results and this is stipulated by organisations such as the World Health Organisation (WHO) and the International Committee of Medical Journal Editors (ICMJE). ICJME stipulates that clinical trials will not be published unless they are registered with a clinical trials registry.

The European Trials Agency (EMA) mandates additional registration with the European Union Drug Regulating Authorities Clinical Trials (EudraCT) database in accordance with the European Clinical Trials Directive 2001/20/EC for all Clinical Trials of Investigational Medicinal Products (CTIMPs).[271] The trial was registered with the EudraCT database following ethics committee approval of the first version of the protocol and given a unique identification number, EudraCT 2013-004205-89. No additional ethics, regulatory or other approvals were required to undertake this thesis.

6 Trial Management

6.1 Study coordination

The UK HARP-III trial was coordinated by the CCO based at CTSU, University of Oxford. The University of Oxford was the trial sponsor (Figure 13). Oversight of the trial and its progress was provided by the Trial Steering Committee (TSC) and safety and efficacy data were closely monitored by an independent Data Monitoring Committee (DMC; Figure 13).

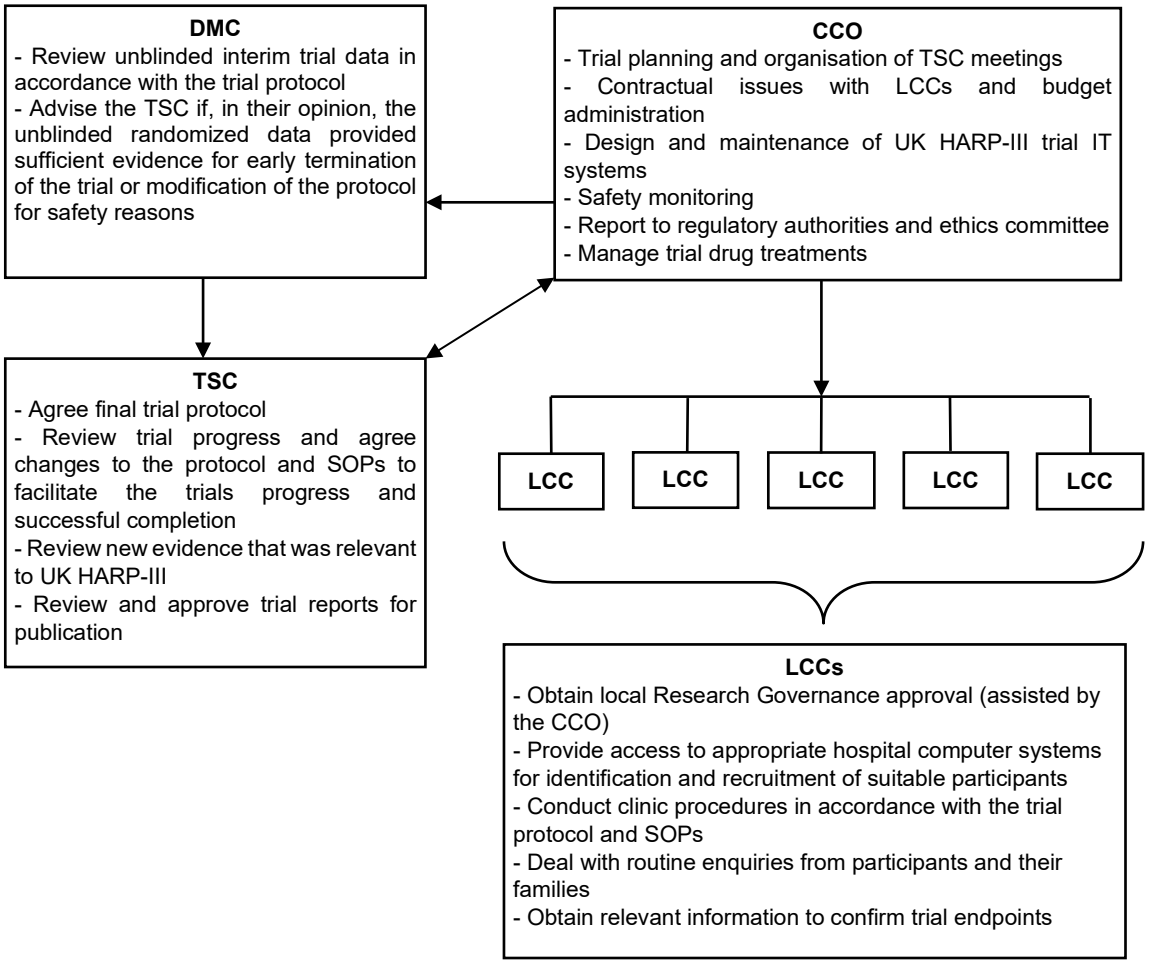


Figure 13: Trial coordination structure
CCO = Central coordinating office; DMC = Data Monitoring Committee; LCC = Local Coordinating Centre; SOP = Standard Operating Procedure; TSC = Trial Steering Committee

6.2 Central coordination of the UK HARP-III trial

The conduct of a large, multicentre trial like UK HARP-III, requires a multi-disciplinary team including amongst others: clinicians, trial managers, administrators, nurses, statisticians, and computer programmers.

The CCO was responsible for overall trial management, establishing local trial sites, ensuring the trial protocol was implemented correctly, data collected were complete and accurate and, that the results were analysed in accordance with the published trial DAP. The CCO carefully monitored the safety of all trial participants and CCO clinicians provided advice to healthcare staff regarding their clinical management and safety concerns. CCO staff managed the delivery of supplies of all study equipment including study treatments, central sample collection kits, blood pressure machines and all study documentation.

I had a central role within the UK HARP-III CCO team and worked closely with the Chief Investigator of the trial, Professor Richard Haynes and, the two Principal Investigators, Professor Colin Baigent and Professor Martin Landray, who maintained overall responsibility for the trial. The Chief Investigator was responsible for the design and overall conduct of UK HARP-III.

I helped prepare documents for regulatory approvals and supported and supervised the trial administrative team with setting up local study sites. I liaised with nephrologists, local research and development staff, pharmacists and nurses at these local sites to resolve issues that arose during site set-up (and throughout the trial) to ensure that the trial could be undertaken at the site.

6.2.1 Trial documentation

The CCO generated all study documents required for conducting the trial. The CCO supplied local sites with all documents and materials at the start of the trial prior to recruitment beginning at the site. Additional materials required to conduct trial procedures and tasks were provided throughout the trial as required.

6.2.1.1 *Trial protocol*

The Chief Investigator was responsible for preparation of the protocol and all subsequent revisions. I assisted with refinements and revisions of the trial protocol, the final version of which was approved by the TSC.

During the recruitment phase, the trial eligibility criteria were reviewed by the TSC and amended, to improve the number of patients that were eligible for the trial and the overall rate of recruitment. Many patients that were expected to be eligible for the trial based on previous renal function and proteinuria results were failing at the screening visit due to ineligible lab results such as a potassium greater than 5.2 mmol/L (for example due to a haemolysed sample) or albuminuria results that were slightly out of range for eligibility (having previously been expected to be in the range for eligibility). The TSC agreed the following changes to the protocol to increase the number of patients eligible at screening:

- Individuals with an eGFR of 20 to 45 mL/min/1.73m^2 were eligible regardless of the level of albuminuria (previously all participants were required to have a uACR greater than 20 mg/mmol)
- Individuals with an eGFR of 45 to 60 mL/min/1.73m^2 were only eligible if they also had a uACR greater than 20 mg/mmol
- potassium criteria for exclusion at screening changed from 5.2 to 5.5 mmol/L

Additionally, some participants who were eligible at screening became ineligible at randomization as their blood pressure had fallen below 130/80 at the visit. In many cases this was due to over-treatment of blood pressure during the run-in phase when RAS inhibitors had been withdrawn. To avoid drop-out at randomization, the blood pressure criteria for eligibility at randomization were modified so participants remained eligible unless systolic blood pressure was below 110 mmHg at randomization in asymptomatic patients (or below 130/80 if participants were symptomatic at this level).

In June 2015, new data emerged suggesting sacubitril/valsartan may take up to 9 months to have full effect on renal function.[200] The TSC agreed an extension in the duration of trial follow-up from 6 to 12 months. Ethics committee and regulatory approval was obtained, and all participants were provided with an updated PIL explaining the rationale for the extension to trial follow-up and invited to continue the trial for an additional 6 months. All participants agreeing to the extended follow-up were re-consented to formally document their agreement to extended follow-up. The TSC also provided comments and approvals for all trial publications including the trial rationale/baseline and main results publications.[255,272]

6.2.1.2 *Standard Operating Procedures and internal operating procedures*

Under the guidance of the Chief Investigator, I produced the Local Clinical Centre Manual of Operations which outlined the study protocol, providing detailed

descriptions of all trial procedures to enable LRCs at LCCs to perform all trial visits and procedures. The manual included a guide on how to perform actions in the trial database, *Cello*.

I drafted an SOP outlining the procedures for sample collection, transport, storage and central analysis of blood and urine samples collected at local sites. I provided feedback on other trial SOPs including the reporting of adverse events.

I wrote the Data (or Statistical) Analysis Plan (DAP) for the trial with a team of statisticians and clinicians (Appendix 1: Supplementary material).[272] The DAP was approved by the TSC and published prior to the unblinding of any trial participants and, before any unblinded analyses were performed.[272] Statisticians at the coordinating centre provided all statistical support for the trial and performed all trial analyses.

I wrote an internal operating procedure (IOP) outlining the responsibilities of trial clinicians working on the trial including daily review of all adverse events and laboratory results, dealing with LRCs and patient queries and, procedures for unblinding trial participants if necessary.

6.2.1.3 *Participant-facing documents*

I was responsible for drafting several of the participant-facing trial documents prior to submission for the necessary regulatory approvals and throughout the trial, including the study poster (Appendix 3), PIL (Appendix 4), study treatment information sheet (STIL; Appendix 5), participant reminder card (Appendix 7) and study participation card (Appendix 8). I provided feedback on the participant letters to GPs and their local nephrologists, which informed the responsible clinician of the participants' involvement in the trial. I worked closely with the Chief Investigator to draft, review and revise all trial documentation.

6.2.2 Local clinical centres (LCC)

I travelled to several LCCs to address any issues local staff had with establishing the LCC (for example, concerns relating to issuing study treatment). The visits increased awareness of the trial amongst clinical staff and encouraged participant recruitment through the use of the pre-screening method.

As an LCC clinician for Oxford University Hospitals NHS Foundation Trust, I worked closely with the LRCs to recruit patients and to provide clinical support for all follow-

up visits when required. I provided clinical advice throughout the trial, for example, on BP management, abnormal laboratory results and assisted with collecting blood samples for mGFR tests.

6.3 Participant recruitment

During the recruitment phase, weekly emails were sent to local sites to monitor numbers of participants pre-screened, invitations sent out and the response rates from potential participants. This regular contact encouraged LRCs to continue to recruit patients and provided an opportunity to offer additional assistance if needed during the recruitment phase. The weekly emails allowed sites with slower recruitment rates to be identified much earlier and provide them with any additional support more swiftly.

Sites with poor recruitment rates (determined by numbers of patients invited and registered in the study database at monthly intervals) were contacted regularly and recruitment was discussed with the study nurses and LLIs to resolve any concerns regarding recruitment. At some sites, study nurses found it difficult to find time to make the follow-up telephone calls to patients after invitations were sent out, particularly at sites where nurses worked less than full-time or if the research nurse was single-handedly coordinating the trial).

All sites were offered additional funding to cover a research assistant to support LRCs with making initial telephone calls to patients to ask whether they were interested in participating in the trial. This reduced the number of calls study nurses needed to make to patients by removing this element of their workload. Sites that utilised this resource found that their recruitment rates improved trial efficiency.

If despite these additional supportive measures being implemented sites could not recruit (or recruit adequate numbers) of participants, they were closed prior to randomizing any participants. Enrolling fewer sites that were able to recruit larger numbers of participants prevented the quality of the trial data at the site being compromised and avoided the costs of monitoring multiple sites with small numbers.

To further encourage LRCs to utilise pre-screening and to improve nurses' confidence with the method, I helped produce a video entitled 'Transforming Renal Trial Recruitment'. The short video demonstrated the successful implementation of pre-

screening in a large international cardiovascular trial.[273] The video outlined what pre-screening entails, the experiences of two UK HARP-III trial participants and a research nurse describing her experience of the trial and pre-screening. The video included feedback regarding pre-screening from other UK HARP-III nurses across the UK. The video helped allay concerns from LRCs regarding telephoning patients after sending invitation letters and, demonstrated how successful and more effective pre-screening was compared with other methods of recruitment.

I drafted a monthly newsletter which outlined new information relating to the trial, study procedures and recruitment progress. At the end of recruitment, I drafted a newsletter that was sent to all randomized participants informing them of trial progress, safety information and what to expect on completion of the trial.

A 'league' table of recruitment by site was included in the monthly newsletters at the request of local research coordinators. Sites were ranked by numbers of patients screened in the preceding month and, sites with the highest recruitment rates were highlighted in the newsletter. The table encouraged sites to recruit larger numbers of participants.

To increase the rate of overall trial recruitment, five additional LCCs were established. Four of these centres were subsequently closed prior to randomization of any participants due to recruitment difficulties.

At the end of the recruitment phase, I produced a questionnaire to collect information from all local clinical centres (Appendix 6) about the methods of recruitment used to recruit participants for UK HARP-III. Information was collected on the catchment size of the population covered by the local renal unit, the principal method(s) for identifying potentially eligible patients and whether follow-up phone calls were made. The responses enabled analysis of the utility of 'pre-screening' compared with 'traditional' methods of recruiting trial participants (e.g. consultant referrals or manually searching clinic lists) in future large renal trials.

6.4 Leadership

I led the trial team daily to ensure the smooth-running of the trial. I managed all clinical queries from research nurses at local sites and provided advice and support with administrative queries from sites. I supervised and assisted the team with managing

the trial email account and drafting responses to sites as needed. I answered all clinical and some of the administrative telephone queries.

I reviewed weekly study progress reports to ensure that adequate treatment and follow-up compliance was maintained, blood results were entered on time and outstanding trial actions had been completed at sites. I supported the trial team with chasing outstanding tasks and contacting LRCs and LLIs where required. I also reviewed and approved minutes of weekly trial meetings prior to dissemination to the remainder of the CCO UK HARP-III team.

As the trial progressed, I took over leading and running the trial training days for new staff including administrative staff, research nurses and trial monitors. This involved working with a trial administrator to coordinate and organise the agenda, speakers, dates for training and delivering the training. I led the administrative team in preparing mock exercises for the newly trained staff to complete following the training days. Once the individuals had completed the exercises, I reviewed the responses to confirm that they understood the trial procedures and were competent with using the IT system.

I worked with the administrative team to coordinate the retrieval and transport of central blood and urine samples back from local sites to the central laboratory (the Wolfson laboratory) based in Oxford. All central samples were stored and analysed (with the exception of pharmacokinetic analyses) in the Wolfson laboratory.

I assisted with drafting reports for TSC and DMC meetings and drafted abstracts and manuscripts for oral presentations and journal publications relating to the UK HARP-III trial (Appendices 1, 9 and 10).[255,272,274]

6.5 Trial database and data management

The UK HARP-III trial IT system, *Cello*, was a web-based electronic data capture system. The bespoke system was developed by programmers based at the coordinating centre and modelled on databases created for data collection in other large randomized trials led by the coordinating centre. I worked with the Chief Investigator and the IT system leads to design a system that would comply with the trial protocol and clinical requirements to enable participant data to be captured real-time and entered directly into the trial database.

The specification for the design and functionality of *Cello* was written by a computer systems analyst and was updated as dictated by the required functionality of the database. The specification document facilitated the programming and testing of the database prior to the live database being released for use. I attended weekly meetings with the IT team during database development and helped with testing prior to the database going live. I identified and reported issues with the functionality of the database that required fixing throughout the trial.

The web-based system enabled direct data entry into *Cello* during study visits. The trial protocol did not permit any data to be recorded on paper (or elsewhere) and transcribed into the database at a later time. Direct data entry had several advantages: it allowed checks at the time of data entry to avoid data transcription errors improving the reliability of the trial data; ensured that the study database and data collection was as complete as possible at the end of the trial and; improved efficiency with running the trial.

All access to the database required a unique electronic username and password, and all new information and any changes to existing data required the user to enter their username and password as an electronic signature. This ensured an audit-trail could be maintained and monitored and so only those individuals with a *Cello* account could access confidential participant data.

Access within *Cello* was restricted according to an individual's role in the study both at the coordinating centre and at local sites. For example, CCO clinicians could review the full breadth of clinical data including laboratory results, adverse events, visit forms and treatment records. However, CCO administrators could not access any clinical information. Similarly, at local sites, only the LLI and any designated clinicians with overall responsibility for participants could approve participants for randomization. A web-based system provided flexibility with performing study visits as they could be performed from any location with internet access.

Research nurses managed all aspects of the trial using *Cello*, including booking study visits, completion of case report forms, entering local laboratory and mGFR results, recording adverse events and issuing study treatment. A task list was built into *Cello* to remind LRCs of outstanding tasks for participants. The task list enabled me and CCO administrators to monitor outstanding actions at sites. A task would not be removed from the list of outstanding actions until it had been completed (for example, a follow-up appointment made, or blood results entered).

Cello allowed CCO staff to perform a wide range of trial procedures including monitoring and ordering of drug supplies for sites, reviewing safety parameters (such as adverse event data and local laboratory results), monitoring reports of compliance with study treatment and follow-up. The outstanding actions and participant progress reports helped ensure study visits occurred within the specified timeframes, for example before participants ran out of study treatment.

6.6 Teaching and training

Before recruitment could begin, all local coordinating centre received training with performing study procedures and using *Cello*. I had a central role in the organisation and delivery of the training.

I led the one-day training sessions at the CCO which educated staff about the UK HARP-III trial rationale and methodology. I arranged training dates, organised speakers and delivered much of the teaching to research staff (including doctors, nurses, study monitors and administrators). Throughout the trial, additional 'refresher' training was available if required, both on-site at the CCO and remotely using web-based conferencing.

A series of short presentations were delivered, covering:

- rationale, design and conduct of the UK HARP-III trial, as described in the protocol and this document
- eligibility criteria and recruitment method
- study treatment handling, distribution and storage
- general features of clinical trials, including discussion of International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines and the practical procedure for obtaining valid informed consent
- collection, processing and transfer to the coordinating centre of samples for central analysis
- adverse event reporting
- administrative information (e.g. documentation, finance, local coordinating centre site maintenance)

The presentations were followed by practical demonstrations of the IT system, *Cello* and how trial procedures are performed within *Cello*. Copies of all training slides were provided to the research staff and were available in *Cello* for reference. Study nurses were provided with laptops on which they could familiarise themselves with *Cello* using a demonstration version of the system. These 'practical' sessions were led by me and other CCO clinical staff working on the UK HARP-III trial.

I developed a set of training exercises that local research staff were required to complete following the training session to demonstrate they understood the study procedures and were competent in using *Cello*. Test/dummy patients were created in the demonstration version of *Cello*. A range of trial activities were assessed including receipt of study drug, performing follow-up visits, entering laboratory results and reporting adverse events (serious adverse events, suspected serious adverse reactions and non-serious adverse reactions).

On review, if the exercises were not completed satisfactorily, then additional training was provided as deemed necessary. Following satisfactory completion of the training exercises, an electronic signature (username and password) was issued allowing newly trained research staff to use the 'live' version of *Cello*.

During the recruitment phase, regular teleconferences were arranged by the lead CCO monitor and me to provide LRCs with an additional opportunity to gain advice and support with the trial and have non-urgent queries addressed. Local research nurses were also to share their experiences with research nurses from other sites.

Annual meetings were held for LRCs at which they were updated on trial progress, study procedures and any new information to support them with conducting the trial locally (such as information relating to changes in the trial protocol and the importance of follow-up of all participants as per ITT analyses). An annual UK HARP-III collaborators' meeting was held to inform all staff of future plans for the trial and offered an opportunity for all LLIs and LRCs to meet and discuss and/or feedback any concerns relating to the trial.

6.7 Trial monitoring

Several types of monitoring took place in the UK HARP-III trial. CCO staff coordinated central and local monitoring of study data and procedures. A team of research nurses performed all on-site local trial monitoring (on behalf of the CCO) in accordance with the trial Monitoring SOP. The TSC provided monitoring and advice on trial progress and an independent DMC regularly reviewed the unblinded interim analyses of all serious adverse events and trial data including all blood and urine results and compliance with study follow-up visits and randomized treatment.

The key trial team comprised of administrators, clinicians (including myself) and study monitors, attended a weekly meeting to discuss and review: trial progress, issues at LCCs, study amendments, sample collections, supplies of blood kits and study treatments at local sites. The meeting was led by me and the trial manager, Rejive Dayanandran. Central monitoring reports were also reviewed and planning of staff training days, trial newsletters and study meetings were also discussed.

6.7.1 Central monitoring

6.7.1.1 *Recruitment*

Recruitment rates across all sites were closely monitored by the CCO to ensure that recruitment progressed rapidly and to identify any difficulties sites may be having with recruiting patients. A weekly report was generated of numbers of patients screened by sites. The report was reviewed at the weekly trial management team meeting. The web-based system had in-built monitoring reports, one such report allowed ‘real-time’ review of future screening appointments at sites to track recruitment progress. The weekly emails sent to all sites requesting recruitment data allowed recruitment tracking so that sites recruiting more slowly could be contacted to discuss any concerns or difficulties they may be experiencing.

Copies of all signed participant consent forms were returned to the CCO for review by CCO nurses for any irregularities or errors. Sites were informed of any errors and requested to make corrections to the consent forms as required. The lead CCO monitor for UK HARP-III held regular meetings with local site monitors to discuss any concerns and issues at sites, to plan monitoring visits and discuss trial progress.

6.7.1.2 *Key data*

Sites were asked to provide paper copies of all baseline and final mGFR results to allow verification of the primary outcome data. The result entered into *Cello* was checked against the paper report and any data errors were recorded as a data query for correction at the end of the trial, prior to data analyses and unblinding of participant data. Common errors in mGFR results included entry of results not adjusted for body surface area and incorrect procedure dates.

6.7.1.3 *Compliance*

The web-based data capture system produced reports to follow participants' progress at each site and the reports were reviewed weekly. The reports provided information on a participants' remaining drug supply, and helped CCO staff to ensure compliance with study procedures, including: booking study visits at scheduled times (before drug supplies ran out), compliance with study visits, issuing of study drugs and compliance with study medications and laboratory and mGFR results entry. The reports were linked to the 'task-list' in *Cello* and helped prevent follow-up visits being missed, thereby maintaining compliance with follow-up and randomized study treatment. If a participant's follow-up method changed (for example, to follow-up by telephone or through medical records) then the reports prompted CCO staff to contact LCC staff when a face-to-face study visit would have been scheduled, to collect and upload the available data. I regularly reviewed these reports to ensure that no participant follow-up was missed, particularly for those participants whose follow-up method had changed.

In order to track compliance with study treatment, I maintained a list of all participants stopping study medications either temporarily or permanently, including the reasons for stopping. The lists enabled me to prompt and encourage LCC staff to re-introduce study treatments in those participants in whom it was appropriate to do so. This helped prevent large numbers of participants stopping randomized treatment completely, which would have had a significant impact on study power. Wherever possible, study nurses were encouraged to restart full dose study treatments, if this was not appropriate (or possible) then participants remained on the lower (half) dose of study treatment.

At the time of final follow-up, additional reports were generated to ensure that final study visits and mGFR tests were scheduled and performed on time (specifically before the participant ran out of study treatment). The report identified outstanding

mGFRs results that were not recorded in the study database or for which the paper result had not been received.

6.7.1.4 *Data errors*

Documentation of all data errors and anomalies such as laboratory results being recorded with incorrect dates or values, were maintained within a 'data queries' spreadsheet. All queries were reviewed by CCO clinical staff and a decision was made as to whether a data correction was required. At the end of the trial a bespoke software programme ("ERATO") developed by CCO programmers, was used make any required data corrections. For example, all mGFR results entered in the study database were compared against the paper copy of the report and any errors were reported in the data queries spreadsheet with a link to the correct information (and supporting evidence), creating an audit trail. This process occurred prior to the unblinding of any participant data or study analyses being performed).

6.7.2 Local on-site monitoring

Shortly after local sites began to recruit and screen patients, an on-site monitoring visit was arranged by a CCO trial monitor. The purpose of this visit was to help staff to resolve any local problems with the trial, ensure the trial was being conducted in accordance with the trial protocol and to check the accuracy, completeness and quality of the data entered into *Cello*. In UK HARP-III, as in other trials led by the CCO, trial monitors sat in on and observed trial visits to ensure that the well-being of participants was being respected and that GCP and regulatory standards were being complied with, for example by reviewing the consent-taking process. The clinical trial monitors checked paper copies of local laboratory results, mGFR results and participant data against that recorded in the web-based system to verify the accuracy of the data recorded in *Cello*, as this data was the source data for the trial.

At site monitoring visits, trial monitors visited the local hospital pharmacy to review the conditions where study treatments were stored, ensure that drug issuing procedures were being complied with and, an accurate drug accountability and temperature logs were being maintained.

The trial monitor prepared a report following the monitoring visit and submitted it for review by senior CCO staff. The timing and frequency of further monitoring visits were arranged according to the results of both central and local monitoring of all the trial

data for each site. The site monitors and lead CCO monitor voiced their concerns to the Chief Investigator and to me if there were discrepancies in the delivery of the trial at LCCs. For example, concerns regarding the timing of entry of blood results in relation to completion of a study visit form and whether the results were 'genuine' or if the timeline or sequence of events was plausible to be in keeping with the stipulated trial procedures.

6.7.3 Clinical and participant safety monitoring

6.7.3.1 *CCO monitoring*

I led the management of the clinical and safety monitoring of participant results with assistance from other CCO clinicians. All laboratory results and adverse events recorded in *Cello* were reviewed daily, blinded to treatment allocation. All significant clinical safety events were reported to and discussed with the Chief Investigator and sites were provided with the appropriate advice on management.

All local laboratory results required entry in *Cello* within 48 hours of a trial visit being completed and were reviewed and 'signed-off' by a CCO clinician using a unique electronic signature to verify that they had been reviewed. I signed all results entered in *Cello* on a daily basis. If there were any abnormalities or concerns with the results, then I contacted LCC staff and advised them of the appropriate course of action to be taken. For example, if there were any abnormal potassium or creatinine results, I contacted LRCs by email and advised them on the timing of repeat samples and the management of the abnormal result. For any results needing more urgent action (for example, serum potassium greater than or equal to 6.0 mmol/L or significant rises serum in creatinine), LRCs were contacted by telephone and advised on the management and a follow-up email documenting the advice was sent. I maintained a list of all abnormal results and regularly updated these once results had been repeated, to ensure the abnormal results had been actioned and resolved.

If any irregularities in laboratory results were seen for example, suspected implausible results or values, then these were queried with the local site and a data query recorded where appropriate. I reviewed all such data queries and any concerning patterns of abnormalities were raised with the monitoring team and discussed with the local site as required.

I reviewed all trial visit follow-up forms to monitor participants' blood pressure during clinic visits. If mean blood pressure rose above targets (as per the current KDIGO guidelines on management of blood pressure in CKD)[59] or appeared unusually low, LRCs were advised to discuss with their LLI or the participants' usual nephrologist, particularly if any home blood pressure readings were of a similar range. The management of blood pressure was left to the discretion of local nephrologists however, I (and other CCO clinicians) provided advice if requested or required, in particular to prevent study treatment from being withheld, reduced or withdrawn unnecessarily. This also provided an opportunity to check that participants requiring re-supply of randomized treatment had this re-issued correctly.

I reviewed all reported adverse events (serious and non-serious) daily. No further information was sought for serious adverse events *not* believed to be related to study treatment unless the event was reported as life-threatening or resulted in death. No additional information was sought for non-serious adverse reactions unless deemed a potential event of particular interest, for example a fall which may have resulted from postural hypotension caused by study treatment.

All suspected serious adverse events required discussion with a CCO clinician prior to being entered into the study database. I contacted all LRCs to collect additional detailed information on all suspected serious adverse event reports, including a thorough history of the event, results of any relevant investigations and, current medication history so that a report of the event could be compiled. All suspected serious adverse event reports were discussed with the Chief Investigator and sent for review by the DMC unblinded to study treatment allocation. All clinicians and staff working on the trial remained blind to randomized treatment allocation.

I dealt with all clinical queries from local sites and study participants and provided advice as required. Less urgent clinical queries were received and managed by email and urgent queries were addressed by telephone. During working hours, LRCs could contact the study office with any clinical (or administrative) concerns that they wished to discuss with a clinician. For queries outside office hours or those from participants, the CCO provided a 24 hour, 7 days per week clinical service via a freephone telephone contact that was covered by a clinician at all times, to support all sites, healthcare staff and study participants. I wrote a short guide to assist clinicians that did not routinely work on the UK HARP-III trial. The guide covered the management of significant clinical events of relevance such as the management of symptomatic hypotension, angioedema or hyperkalaemia.

6.7.3.2 *Independent DMC monitoring*

The independent DMC regularly reviewed all the *unblinded* interim analyses of all serious adverse events and trial data including all blood results and compliance with follow-up and trial treatment. In light of these analyses and any other information considered relevant, the DMC provided advice to the TSC if, in their view, the randomized comparisons in the study provided both (i) “proof beyond reasonable doubt” that for all, or some specific types of, patients use of sacubitril/valsartan was clearly indicated or clearly contraindicated; and (ii) evidence that might be reasonably expected to influence materially the patient management of many clinicians who were already aware of the results of other relevant trials.

It was the responsibility of the TSC to then decide whether to modify the trial or to seek additional data (where relevant). Unless this happened, the TSC, collaborators, trial participants, and all trial staff (except those who provided the confidential analyses to the data monitoring committee) remained blinded to these interim analyses.

7 Results

7.1 Recruitment

7.1.1 Recruitment method

At the end of the recruitment phase, local sites were sent a recruitment questionnaire to complete (Appendix 6). Sites were asked to report their primary method of recruitment, any alternative methods used to recruit, the size of the catchment area served by their renal unit and the number of CKD patients cared for by their unit.

Sites recruiting participants using the preferred method of recruitment for UK HARP-III as recommended by the CCO (i.e. pre-screening potentially eligible participants using electronic hospital databases, sending out batches of invitations and telephoning patients about a week later) recruited larger numbers of participants and, more rapidly than sites using 'traditional' methods of recruitment (e.g. awaiting consultant referrals or approaching patients attending renal clinics; Figure 8).

Of the 24 UK HARP-III sites, 13 sites (total catchment population 11.1 million people) used the pre-screening method for recruitment and 11 sites (total catchment population 15.3 million people) primarily used traditional methods of recruitment. The median (IQR) screening rate was 21.9 (13.5-40.4) participants per million population per year (pmp py) at sites primarily using pre-screening methods compared with 14.1 (8.4-27.4) pmp py at sites primarily using traditional recruitment methods (P=0.20 by Mann-Whitney U test).

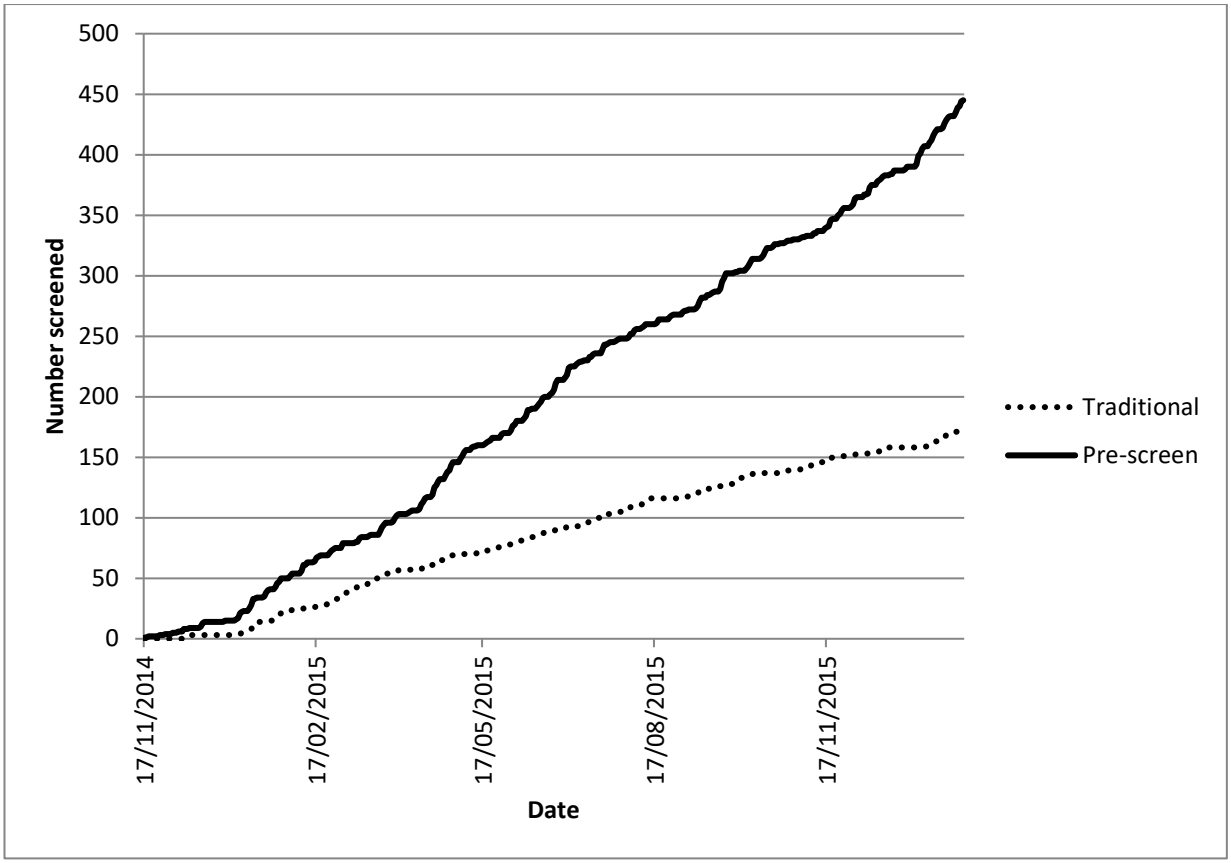
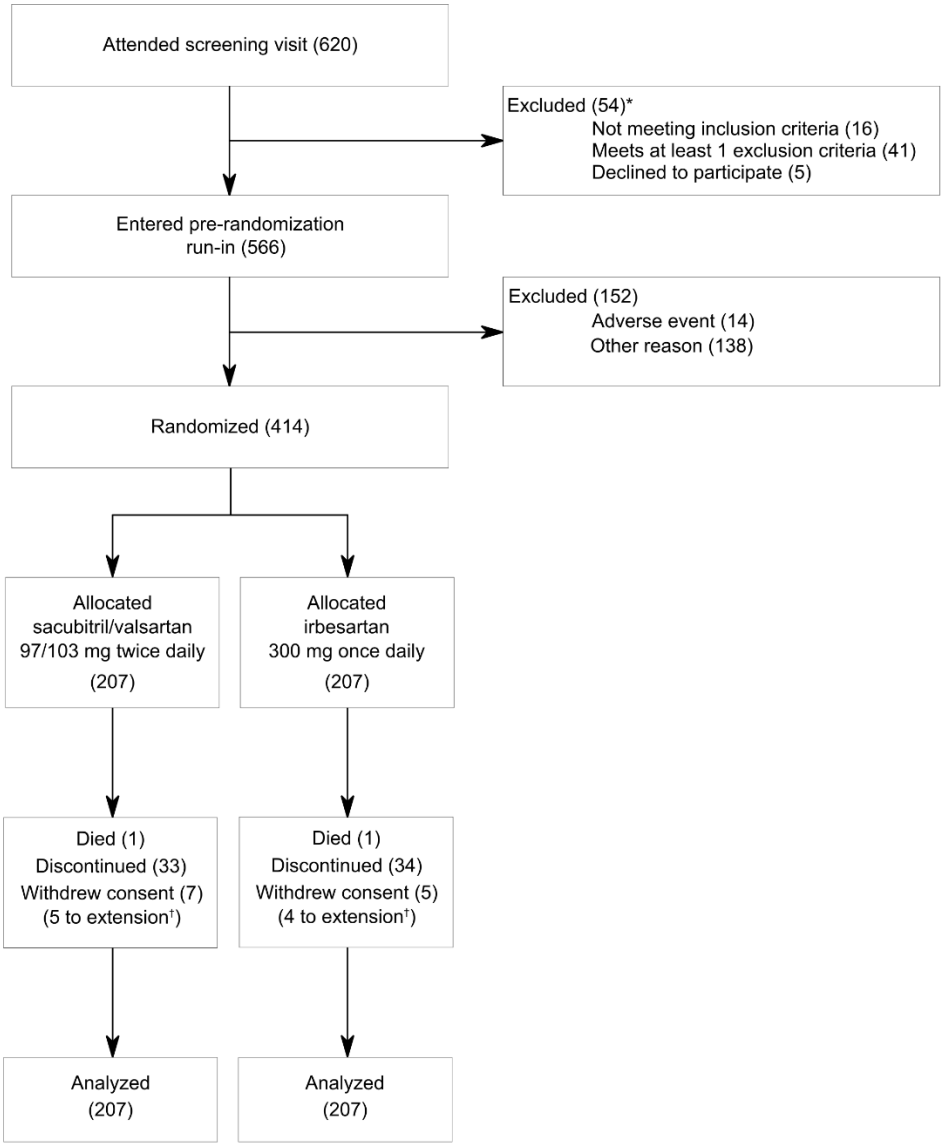


Figure 8: Numbers of participants screened using the pre-screening and traditional methods of recruitment

Between November 2014 and March 2016, 620 patients were screened at 24 sites across the UK. Of the 620 participants screened, 566 (91%) entered the pre-randomization single-blind placebo run-in phase (Figure 9).



*Participants could report more than one reason
†The duration of the trial was increased from 6 to 12 months and 9 participants did not consent to this extension so completed follow-up at 6 months.

Figure 9: Flow of participant through the UK HARP-III trial

7.1.2 Ineligibility at screening

54 participants were ineligible at the screening visit. The reasons for ineligibility are outlined in Table 10. The main reason for ineligibility was out of range historic laboratory results, particularly potassium and uACR. Due to the drop-out at screening, the TSC amended the potassium threshold for exclusion from greater than 5.2 mmol/L to greater than 5.5 mmol/L in March 2015 (Table 10).

Reason for ineligibility during the Screening visit*	N (%)
Ineligible medical history	
Contraindication to angiotensin receptor blocker	5 (9%)
Previous adverse reaction to angiotensin receptor blocker	3 (6%)
On immunosuppression for nephrotic syndrome	4 (7%)
Known chronic liver disease	4 (7%)
Previous angioedema	6 (11%)
Taken an unlicensed investigational medicinal product in last month	1 (2%)
Medical condition that might limit the participant's ability to take study treatment for the duration of the study	11 (20%)
Ineligible based on laboratory measurements available at the time of the screening visit	
eGFR out of range	7 (13%)
uACR ≤20 mg/mmol (or uPCR ≥30 mg/mmol)	10 (19%)
Potassium too high**	11 (20%)
Serum albumin <<30 g/L and uACR >300 mg/mmol (or uPCR >350 mg/mmol)	2 (4%)
Alanine or aspartate transferase 2 x the upper limit of normal	1 (2%)
Ineligible based on blood pressure at the screening visit	
Mean systolic blood pressure >180 mmHg	5 (9%)
Declined to consent	5 (9%)
Total ineligible at screening visit	54

Table 10: Reasons for ineligibility for UK HARP-III during the screening visit
eGFR = estimated glomerular filtration rate; uACR=urinary albumin:creatinine ratio; uPCR=urinary protein:creatinine ratio. *More than one reason may apply.
**Threshold for exclusion for potassium was changed from >5.2 to >5.5 in March 2015 (protocol version 6).

7.2 Pre-randomization run-in

138 participants were withdrawn from the pre-randomization run-in phase prior to attending the randomization visit (reasons for withdrawal are outlined in Table 11). The most common reason for withdrawal from run-in was blood and urine samples taken at the screening visit not meeting the eligibility criteria in 43% of cases (Table 11). Blood pressure related adverse events were not a cause of withdrawals from run-in. Participants thought unlikely to tolerate or comply with study medications and/or scheduled study visits for the duration of the trial were encouraged to withdraw (Table 11).

Overall, 152 (27%) of the 566 participants that entered the pre-randomization run-in phase were not eligible to proceed to randomization.

Reason for withdrawal from placebo run-in*	N (%)
Adverse event	
SAE (unrelated)	3 (2%)
NSAR	7 (5%)
Subtotal: Any adverse event	10 (7%)
Participant died during run-in**	1 (1%)
Other reason	
Ineligible screening lab result	59 (43%)
Unable to attend clinic	5 (4%)
Concerns about tablets	13 (9%)
Difficulty taking tablets	3 (2%)
Doctor advice	13 (9%)
Trial administration problem	11 (8%)
Undergoing investigations	1 (1%)
Family circumstances	1 (1%)
Travel problem	5 (4%)
Patient wishes	16 (12%)
Subtotal: Any other reason	127 (92%)
Total drop-out during run-in	138

Table 11: Reasons for withdrawal from pre-randomization run-in
NSAR=non-serious adverse reaction; SAE=serious adverse event.
*More than one reason may apply. **This participant died of pneumonia.

Four participants were withdrawn from run-in due to a serious adverse event (including myocardial infarction, septic shock and two cases of pneumonia [one of which was fatal]). These events were not related to run-in study treatment (Table 12).

Seven participants reported non-serious adverse reactions believed to be related to the placebo run-in study medication. Individuals reporting non-serious adverse reactions were not eligible for randomization.

Overall, adverse events during run-in were uncommon. 7% of participants experienced at least one adverse event during a mean of 33.7 person-days of follow-up during run-in (Table 12). Most adverse events were due to non-cardiovascular causes.

Adverse events during Run-in	Placebo run-in
Entered run-in	566
Total person-days follow-up	19065
Mean person-days follow-up	33.7
Cardiovascular causes	
Stroke	0 (0%)
Transient ischaemic attack	0 (0%)
Other vascular hypertensive disorders	6 (1%)
Cardiac event	1 (0%)
Other vascular	1 (0%)
Subtotal: Any cardiovascular	9 (2%)
Non-cardiovascular causes	
Cancer	0 (0%)
Infection	4 (1%)
Respiratory	3 (1%)
Other non-cardiovascular	24 (4%)
Subtotal: Any non-cardiovascular	29 (5%)
Total: Any adverse event	37 (7%)

Table 12: Any adverse event during pre-randomization run-in

7.3 Ineligibility at randomization

428 patients attended the randomization visit and 14 were ineligible for randomization (reasons outlined in Table 13). The commonest reason for this was mean systolic blood pressure falling below the threshold for inclusion at randomization (below 130 mmHg).

Reasons for ineligibility at randomization*	N (%)
Consent not confirmed	0 (0%)
Compliance ≤80%**	0 (0%)
Mean systolic blood pressure too low†	9 (64%)
Mean systolic blood pressure >180mmHg$	1 (7%)
Female ≤55 years and unwilling to use reliable contraception	0 (0%)
Medical History	
Related adverse event reported (serious or not)	3 (21%)
Contraindicated medication started	1 (7%)
Total drop-out at randomization visit	**14**

Table 13: Reasons for ineligibility at randomization
*More than one reason may apply.
**Compliance was estimated from direct questioning of participants and pill counts were not performed. Compliance of 80% or less was classified as having taken study treatment on at least six days per week on average.
†Threshold changed in March 2015 (protocol version 6). $Removed in March 2015 (protocol version 6).

7.4 Baseline characteristics

414 participants were randomized into the UK HARP-III trial: 207 to sacubitril/valsartan and 207 to irbesartan. Baseline characteristics were similar between the two groups (Table 14).

The mean age at randomization among the whole cohort was 62.8 (SD 13.7) years. Participants allocated sacubitril/valsartan were slightly younger than those allocated irbesartan (mean [SD] age 62.0 [14.1] versus 63.6 [13.4] years respectively). 72% were male and 91% of participants were white (Table 14). Representation from ethnic minority groups was low with only 4% South Asian, 2% black and 3% of participants from other ethnic groups.

Overall, 40% of participants reported having diabetes mellitus, 13% coronary heart disease, 7% cerebrovascular disease and 4% heart failure at randomization (Table 14A&B).

	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	All participants (n=414)
Demographics			
Age at randomization (years)	62.0 (14.1)	63.6 (13.4)	62.8 (13.7)
<50	37 (18%)	36 (17%)	73 (18%)
≥50 to <70	97 (47%)	99 (48%)	196 (47%)
≥70	73 (35%)	72 (35%)	145 (35%)
Sex			
Male	148 (71%)	150 (72%)	298 (72%)
Female	59 (29%)	57 (28%)	116 (28%)
Ethnicity			
White	186 (90%)	191 (92%)	377 (91%)
Black	3 (1%)	4 (2%)	7 (2%)
South Asian	11 (5%)	7 (3%)	18 (4%)
Other	7 (3%)	5 (2%)	12 (3%)
Medical history			
Prior disease			
Coronary heart disease	21 (10%)	33 (16%)	54 (13%)
Cerebrovascular disease	16 (8%)	15 (7%)	31 (7%)
Peripheral vascular disease	22 (11%)	22 (11%)	44 (11%)
Heart failure	8 (4%)	7 (3%)	15 (4%)
Diabetes mellitus	81 (39%)	83 (40%)	164 (40%)
Renal diagnosis*			
Cause of kidney disease			
Glomerular disease	60 (29%)	51 (25%)	111 (27%)
Tubulointerstitial disease	18 (9%)	32 (15%)	50 (12%)
Diabetic kidney disease	36 (17%)	47 (23%)	83 (20%)
Hypertensive/renovascular Disease	18 (9%)	24 (12%)	42 (10%)
Other systemic diseases affecting the kidneys	1 (0%)	2 (1%)	3 (1%)
Familial/hereditary Nephropathies	30 (14%)	13 (6%)	43 (10%)
Other known causes	5 (2%)	4 (2%)	9 (2%)
Unknown	39 (19%)	34 (16%)	73 (18%)
Medication history			
Medication			
Antiplatelet therapy	64 (31%)	75 (36%)	139 (34%)
Oral anticoagulant	13 (6%)	15 (7%)	28 (7%)
Diuretic	79 (38%)	85 (41%)	164 (40%)
Calcium channel blocker	104 (50%)	103 (50%)	207 (50%)
Beta blocker	50 (24%)	62 (30%)	112 (27%)
Alpha blocker	58 (28%)	55 (27%)	113 (27%)
LDL-lowering agent	126 (61%)	137 (66%)	263 (64%)
Use of RAS blockade at screening visit			
Yes	173 (84%)	166 (80%)	339 (82%)
No	34 (16%)	41 (20%)	75 (18%)

Table 14A: Baseline characteristic of UK HARP-III participants randomized
Values are n (%), mean (SD), geometric mean (approx. SE) or median (IQR). RAS=Renin-angiotensin system. *Renal diagnoses as per the ERA-EDTA registry.

	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	All participants (n=414)
Physical measurements			
Systolic blood pressure (mmHg)	146 (16)	146 (16)	146 (16)
<140	76 (37%)	85 (41%)	161 (39%)
≥140 to <160	93 (45%)	84 (41%)	177 (43%)
≥160	38 (18%)	38 (18%)	76 (18%)
Diastolic blood pressure (mmHg)	81 (11)	80 (11)	81 (11)
<80	96 (46%)	105 (51%)	201 (49%)
≥80 to <90	68 (33%)	58 (28%)	126 (30%)
≥90	43 (21%)	44 (21%)	87 (21%)
Body mass index (kg/m²)	30 (6)	31 (6)	30 (6)
<25	35 (17%)	33 (16%)	68 (16%)
≥25 to <30	74 (36%)	73 (35%)	147 (36%)
≥30	95 (46%)	100 (48%)	195 (47%)
Not available	3	1	4
Medication history			
Medication			
Antiplatelet therapy	64 (31%)	75 (36%)	139 (34%)
Oral anticoagulant	13 (6%)	15 (7%)	28 (7%)
Diuretic	79 (38%)	85 (41%)	164 (40%)
Calcium channel blocker	104 (50%)	103 (50%)	207 (50%)
Beta blocker	50 (24%)	62 (30%)	112 (27%)
Alpha blocker	58 (28%)	55 (27%)	113 (27%)
LDL-lowering agent	126 (61%)	137 (66%)	263 (64%)
Use of RAS blockade at screening visit			
Yes	173 (84%)	166 (80%)	339 (82%)
No	34 (16%)	41 (20%)	75 (18%)
Laboratory measurements			
CKD-EPI estimated glomerular filtration rate at randomization (mL/min/1.73m²)			
Mean (SD)	35.4 (11.0)	35.5 (11.0)	35.5 (10.9)
<30	79 (38%)	77 (37%)	156 (38%)
≥30 to <45	86 (42%)	91 (44%)	177 (43%)
≥45	41 (20%)	39 (19%)	80 (19%)
Not available	1	0	1
Urine albumin:creatinine ratio at randomization (mg/mmol)			
Geometric mean (approx. SE)	34 (5)	34 (5)	34 (3)
Median (IQR)	52 (11-162)	56 (11-146)	54 (11-153)
<3	30 (14%)	28 (14%)	58 (14%)
≥3 to <30	43 (21%)	45 (22%)	88 (21%)
≥30	134 (65%)	134 (65%)	268 (65%)
24-hour urinary sodium excretion during run-in (mg/24 hours)			
Geometric mean (approx. SE)	2245 (183)	2585 (187)	2400 (132)
Median (IQR)	2484 (1794-3795)	2875 (1932-4232)	2680 (1817-3910)
Not available	100	110	210

Table 15B: Baseline characteristic of UK HARP-III participants randomized
Values are n (%), mean (SD), geometric mean (approx. SE) or median (IQR).
RAS=Renin-angiotensin system. CKD-EPI=Chronic kidney disease Epidemiology Collaboration.

7.4.1 Renal characteristics

The commonest causes of CKD were glomerular disease (27%), diabetic kidney disease (20%) and CKD due to unknown aetiology (18%). Mean (SD) systolic blood pressure was 146 (16) mmHg and diastolic blood pressure 81 (11) mmHg at randomization (between 4 and 7 weeks after withdrawal of prior ACEi or ARB or both). Mean CKD-EPI eGFR was 35.5 (SD 10.9) mL/min/1.73m^2. 38% of participants had advanced CKD with an eGFR less than 30 mL/min/1.73m^2, 43% had an eGFR of 30 to 45 mL/min/1.73m^2 and 19% had an eGFR of 45 to 60 mL/min/1.73m^2.

The geometric mean uACR (approximate SE) was 34 (3) mg/mmol and median (IQR) uACR was 54 (11-153) mg/mmol. 14% of participants had normoalbuminuria, 21% had microalbuminuria and 65% had macroalbuminuria (Table 14). 82% of participants were taking RAS blockade (either ACE inhibitor, ARB or both) at screening.

204 participants provided a 24-hour urine collection (107 participants allocated sacubitril/valsartan and 97 allocated irbesartan). Median (IQR) urinary sodium excretion during run-in (following 4 to 7 weeks without any treatment with RAS blockade) was 2484 (1794-3795) mg/24 hours in participants randomized to sacubitril/valsartan and 2875 (1932-4232) mg/24 hours in those randomized to irbesartan.

7.4.2 Risk of progression of to end stage renal disease

Risk of progression to ESKD was calculated using a validated 4-variable risk equation that utilizes age, sex, eGFR and uACR.[275] The median 5-year risk of progression of ESKD (using characteristics ascertained at randomization) was 16.5%. 62% of participants had a 5-year risk of ESKD greater than 10% (Figure 10).

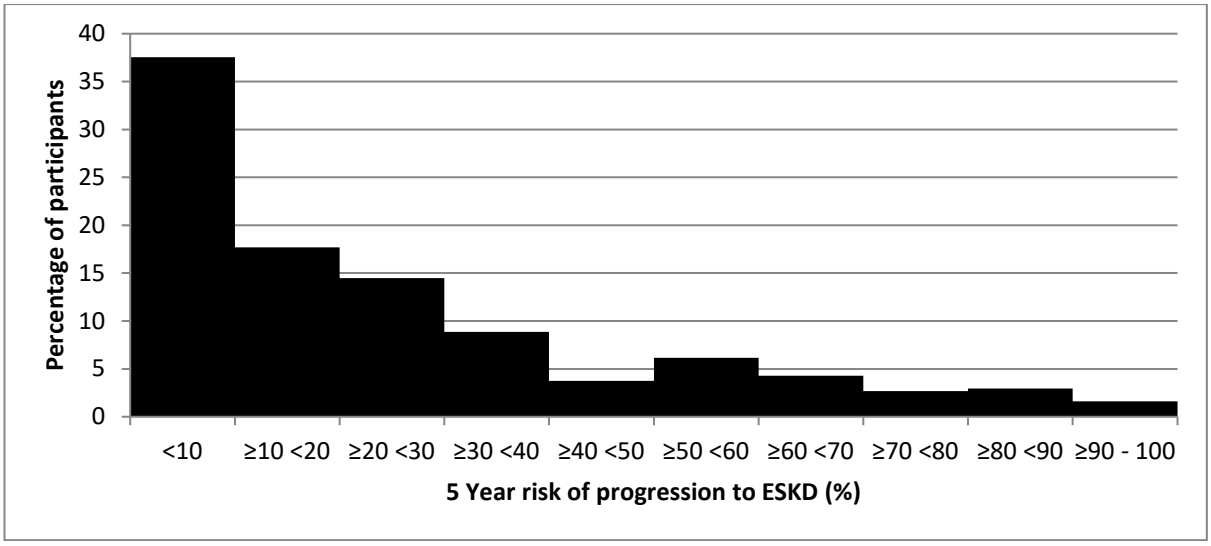


Figure 10: Five-year risk of progression to End-stage kidney disease among UK HARP-III participants

7.5 Compliance with follow-up

Compliance with trial visits was excellent with very few missed follow-up visits (Table 15). If participants were unable to attend in person for a face-to-face visit, then follow-up was completed via telephone and/or using medical records wherever possible.

Scheduled follow-up	No. of participants who started the follow-up period*	Follow-up status			
		Withdrew	Died	Completed**	Missed
1 month	414	0	1	404	9
3 months	412	2	0	408	2
6 months	409	1	0	401	7
9 months	399	2	0	393	4
12 months	397	0	1	394	2

Table 16: Compliance with trial follow-up visits following randomization in UK HARP-III
*Not including those who died or withdrew consent in an earlier follow-up period, or (for the 9- and 12-month rows) the 9 participants who did not consent to extending follow-up from 6 to 12 months.
**Mainly includes survivors who have completed follow-up but may also include those who completed follow-up and subsequently died or withdrew consent for further follow-up during the same period.

At one site, four participants missed the 1-month study visit. On investigation, the participants involved had been reviewed in a trial clinic by the LRCs however, the visit data had not been entered directly into the trial database at the time of the visit. This

constituted a significant study protocol violation and the LRCs received additional training and monitoring. All safety blood and urine samples had been collected and analysed at the time of the visit, so there were no safety concerns for the participants involved because of the 'missed' follow-up visits.

Nine participants declined consent to continue participation in the trial beyond the originally planned six-month trial period following randomization when the trial extended follow-up to 12 months. These individuals had their final mGFR and final follow-up visit at 6 months following randomization.

Only three participants withdrew their consent following randomization for all further follow-up during the trial. Two participants died during follow-up; one due to a pulmonary embolism and the other due to sepsis, neither of these cases were related to randomized treatment.

7.6 Compliance with study treatment

Compliance with study treatments was estimated from direct questioning of participants and pill counts were not performed. Adequate treatment compliance was classified as having taken over 80% of study treatment on at least six days per week on average.

7.6.1 Compliance with full dose study treatment

Compliance with full dose study treatments (either sacubitril/valsartan 97/103 mg twice daily or irbesartan 300 mg once daily) was broadly similar in both groups throughout the trial (Table 16). At three months, compliance with full dose study drugs was 85% in the sacubitril/valsartan arm and 86% in the irbesartan arm. At nine months, this had fallen to 75% in those allocated sacubitril/valsartan and 78% in those allocated irbesartan and at 12 months compliance rates were 76% and 79% respectively (Table 16).

7.6.2 Compliance with any dose of study treatment

Compliance with study drugs (either full dose or less than full dose e.g. half dose of study treatment in those unable to tolerate the full dose) at 3 months post-

randomization was high with 91% of participants taking sacubitril/valsartan and 90% taking irbesartan (Table 16). At final follow-up, compliance with study treatment had fallen to 82% in participants allocated sacubitril/valsartan and 84% in those allocated irbesartan (Table 16).

Scheduled follow-up	No. of participants with scheduled follow-up*	Number (%) taking at least 80% of the full dose of study treatment since last study visit		Number (%) taking at least 80% of any dose of study treatment since last study visit	
		Sacubitril/valsartan	Irbesartan	Sacubitril/valsartan	Irbesartan
1 month	414	172 (83%)	183 (88%)	189 (91%)	195 (94%)
3 months	413	176 (85%)	177 (86%)	188 (91%)	187 (90%)
6 months	411	162 (79%)	169 (82%)	177 (86%)	179 (87%)
9 months	401	150 (75%)	157 (78%)	168 (84%)	169 (84%)
12 months	399	151 (76%)	158 (79%)	164 (82%)	168 (84%)

Table 17: Compliance with randomized treatment in UK HARP-III
*Not including those who died or withdrew consent in an earlier follow-up period, or (for the 9 and 12 month rows) the 9 participants who did not consent to extend follow-up from 6 to 12 months.

7.6.3 Reasons for stopping randomized treatment

Over the 12-month trial duration, 44 (21%) participants stopped sacubitril/valsartan and 42 (20%) stopped irbesartan (P=0.90). Non-serious adverse reactions were the main reason for stopping randomized treatment in both groups (sacubitril/valsartan 24/207 [12%] versus irbesartan 13/207 [6%]; P=0.08; Table 17).

Reason for stopping full dose randomized treatment	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	P Value
Serious adverse event (SAE)			
Angioedema	1 (0%)	0 (0%)	
Hyperkalaemia	0 (0%)	2 (1%)	
Acute kidney injury	1 (0%)	1 (0%)	
Abnormal liver function test	0 (0%)	0 (0%)	
Gastrointestinal disorders	1 (0%)	0 (0%)	
Infections and infestations	1 (0%)	0 (0%)	
Neoplasms benign, malignant and unspecified (including cysts and polyps)	0 (0%)	0 (0%)	
Pregnancy, puerperium and perinatal conditions	0 (0%)	1 (0%)	
Renal and urinary disorders	0 (0%)	1 (0%)	
Respiratory, thoracic and mediastinal disorders	0 (0%)	1 (0%)	
Surgical and medical procedures	0 (0%)	2 (1%)	
Subtotal: Any SAE	4 (2%)	8 (4%)	0.38
Non-serious adverse reaction (NSAR)			
Hypotensive disorder	6 (3%)	2 (1%)	
Hyperkalaemia	4 (2%)	0 (0%)	
Acute kidney injury	1 (0%)	3 (1%)	
Abnormal liver function test	0 (0%)	0 (0%)	
Cardiac disorders	0 (0%)	1 (0%)	
Gastrointestinal disorders	3 (1%)	1 (0%)	
General disorders and administration site conditions	1 (0%)	2 (1%)	
Metabolism and nutrition disorders	1 (0%)	0 (0%)	
Musculoskeletal and connective tissue disorders	1 (0%)	0 (0%)	
Nervous system disorder	4 (2%)	3 (1%)	
Respiratory, thoracic and mediastinal disorders	1 (0%)	1 (0%)	
Skin and subcutaneous tissue disorders	2 (1%)	0 (0%)	
Subtotal: Any NSAR	24 (12%)	13 (6%)	0.08
Other reason			
Unable to attend clinic	2 (1%)	1 (0%)	
Concerns about tablets	0 (0%)	2 (1%)	
Doctor advice	8 (4%)	9 (4%)	
Withdrew consent	5 (2%)	6 (3%)	
Participants wishes	1 (0%)	2 (1%)	
Subtotal: Any other reason	16 (8%)	21 (10%)	0.49
Total: stopped for any reason	44 (21%)	42 (20%)	0.90

Table 18: Reasons for stopping full dose randomized study treatment

Symptomatic hypotension related to study treatment (resulting in a reduction in the dose of trial treatment taken) was more frequently reported in participants randomized to sacubitril/valsartan compared with irbesartan (6 versus 2 cases respectively), as was hyperkalaemia (4 versus 0 cases respectively). However overall, there was no significant difference in reasons for stopping full dose study treatments between the sacubitril/valsartan compared with irbesartan (44/207 [21%] versus 42/207 [20%] respectively; P=0.90).

Similarly, there were no differences in the reasons for participants completely stopping all of their study treatments between sacubitril/valsartan, compared with irbesartan (33/207 [16%] versus 34/207 [16%] respectively; P=1.00; Table 18 [a subset of Table 17]).

Reason for completely stopping randomized treatment	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	P value
Serious adverse event (SAE)			
Angioedema	1 (0%)	0 (0%)	
Hyperkalaemia	0 (0%)	1 (0%)	
Acute kidney injury	1 (0%)	1 (0%)	
Abnormal liver function test	0 (0%)	0 (0%)	
Gastrointestinal disorders	1 (0%)	0 (0%)	
Infections and infestations	1 (0%)	0 (0%)	
Neoplasms benign, malignant and unspecified (including cysts and polyps)	0 (0%)	0 (0%)	
Pregnancy, puerperium and perinatal conditions	0 (0%)	1 (0%)	
Renal and urinary disorders	0 (0%)	1 (0%)	
Respiratory, thoracic and mediastinal disorders	0 (0%)	1 (0%)	
Surgical and medical procedures	0 (0%)	2 (1%)	
Subtotal: Any SAE	4 (2%)	7 (3%)	0.54
Non-serious adverse reaction (NSAR)			
Hypotensive disorder	2 (1%)	2 (1%)	
Hyperkalaemia	4 (2%)	0 (0%)	
Acute kidney injury	1 (0%)	3 (1%)	
Abnormal liver function test	0 (0%)	0 (0%)	
Cardiac disorders	0 (0%)	1 (0%)	
Gastrointestinal disorders	3 (1%)	1 (0%)	
General disorders and administration site conditions	1 (0%)	2 (1%)	
Metabolism and nutrition disorders	1 (0%)	0 (0%)	
Musculoskeletal and connective tissue disorders	1 (0%)	0 (0%)	
Nervous system disorder	3 (1%)	2 (1%)	
Respiratory, thoracic and mediastinal disorders	0 (0%)	1 (0%)	
Skin and subcutaneous tissue disorders	2 (1%)	0 (0%)	
Subtotal: Any NSAR	18 (9%)	12 (6%)	0.34
Other reason			
Unable to attend clinic	2 (1%)	1 (0%)	
Concerns about tablets	0 (0%)	1 (0%)	
Doctor advice	3 (1%)	4 (2%)	
Withdrew consent	5 (2%)	6 (3%)	
Participants wishes	1 (0%)	2 (1%)	
Subtotal: Any other reason	11 (5%)	15 (7%)	0.54
Total: stopped for any reason	33 (16%)	34 (16%)	1.00

Table 19: Reasons for completely stopping randomized study treatment

7.7 Primary outcome: Effect of sacubitril/valsartan on rate of change in measured glomerular filtration rate at 12 months

Mean (SE) mGFR at baseline was 34.0 (0.8) mL/min/1.73m² in the sacubitril/valsartan group and 34.7 (0.8) mL/min/1.73m² in the irbesartan group (Table 19).

A total of 404 baseline mGFR results were used and the 10 missing baseline mGFR values were replaced by the baseline eGFR result. At 12 months, 371 mGFR results were available. A value for the 12-month mGFR was imputed for 41 participants, including the 9 participants that did not consent to continue follow-up beyond 6 months, allowing ITT analyses.

At 12 months mean (SE) mGFR decreased from 34.0 to 29.8 (0.5) mL/min/1.73m² in those allocated sacubitril/valsartan and from 34.7 to 29.9 (0.5) mL/min/1.73m² in those allocated irbesartan: between-group difference in mGFR of 0.1 (SE 0.7) mL/min/1.73m²; P=0.86 (Table 19).

		No. with mGFR value imputed*		Mean mGFR (SE) (mL/min/1.73m²)			
Follow-up visit	No. with mGFR value	Dialysis	Other	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	Difference in means (SE)†	P value
Randomization	404		10	34.0 (0.8)	34.7 (0.8)		
12 months	371	2	41	29.8 (0.5)	29.9 (0.5)	-0.1 (0.7)	0.86

Table 20: Primary outcome: Effect of sacubitril/valsartan on rate of change in measured glomerular filtration rate at 12 months
mGFR = measured glomerular filtration rate. †Values are absolute differences in arithmetic means (SE).

A pre-specified sensitivity analysis excluding all participants with missing 12-month mGFR results (i.e. “complete-case analysis”) was also performed to verify the result observed using multiple imputation. Complete case analysis yielded a between-group difference in means (SE) in mGFR of 0.4 (0.7) mL/min/1.73m² (P=0.55; Table 20).

Follow-up visit	No. with mGFR value	No. with mGFR value imputed		Mean mGFR (SE) (mL/min/1.73m²)		Difference in means (SE)*	P value
		Dialysis	Other	Sacubitril/valsartan (n=207)	Irbesartan (n=207)		
Randomization	404		10	34.0 (0.8)	34.7 (0.8)		
12 months	371	2	0	29.8 (0.5)	30.2 (0.5)	-0.4 (0.7)	0.55

Table 21: Pre-specified sensitivity analysis of the effect of randomization to sacubitril/valsartan on measured glomerular filtration rate as 12 months, excluding participants with missing measured glomerular filtration rate values at 12 months
mGFR = measured glomerular filtration rate. *Values are absolute differences in arithmetic means (SE).

7.7.1 Effect of sacubitril/valsartan on mGFR in pre-specified subgroups

There was no difference in the treatment effect of sacubitril/valsartan on mGFR at 12 months, compared with irbesartan, in a range of pre-specified subgroups (Figure 11).

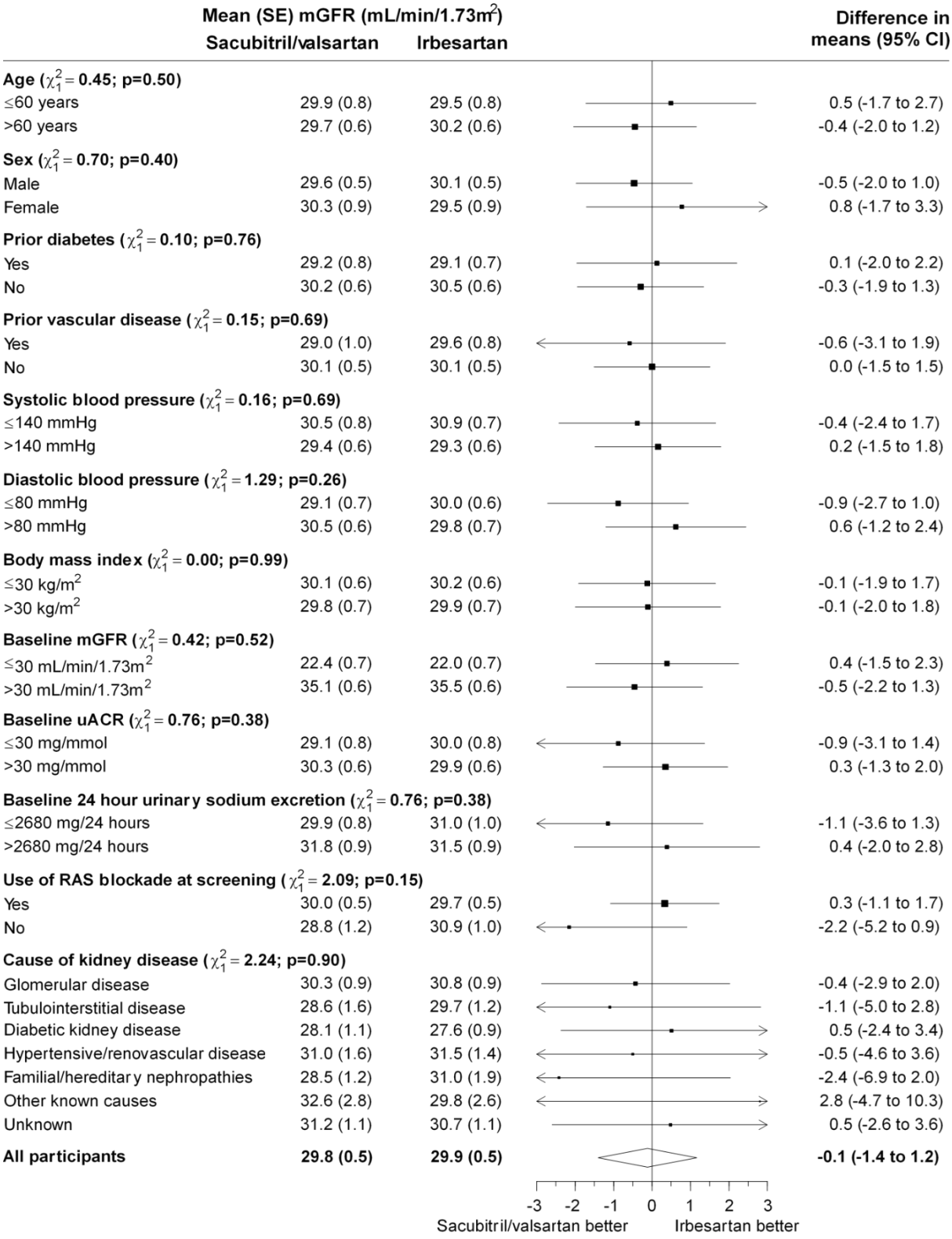
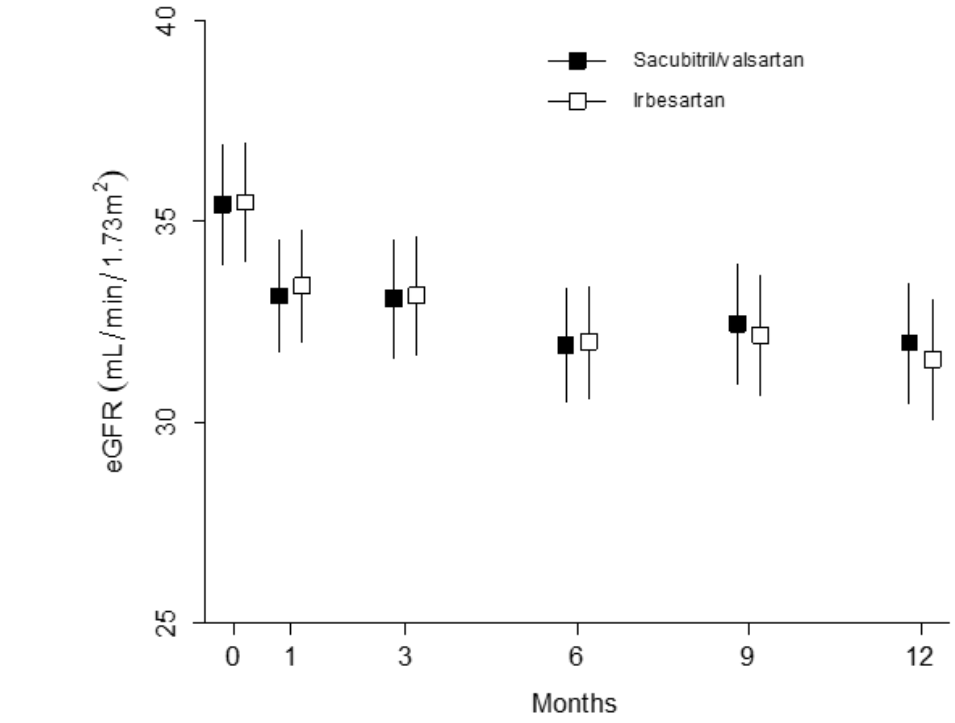


Figure 11: Effect of sacubitril/valsartan on measured glomerular filtration rate at 12 months in a range of pre-specified sub-groups

7.8 Effect of sacubitril/valsartan on secondary outcomes

7.8.1 Effects of sacubitril/valsartan on estimated glomerular filtration rate (eGFR)

eGFR was measured on central samples at randomization, 3, 6 and 12 months and results from local laboratory samples were used for the 1 and 9-month eGFR values. At each time point, there was no difference in mean eGFR between participants randomized to either sacubitril/valsartan or irbesartan (Figure 12). Study average eGFR in participants allocated to sacubitril/valsartan was 32.3 (SE 0.2) and irbesartan 32.2 (SE 0.2) mL/min/1.73m² (difference in means 0.1; 95% CI -0.5 to 0.7; P=0.66).



eGFR (mL/min/1.73m²)						
Sacubitril/valsartan	35.4	33.1	33.1	31.9	32.5	32.0
Irbesartan	35.5	33.4	33.2	32.0	32.2	31.6

Figure 12: Effect of randomization to sacubitril/valsartan on estimated glomerular filtration rate
Error bars presented are 95% CIs.

An acute fall in eGFR was seen on eGFR measurements at one-month post-randomization. This fall reflects the renal haemodynamic changes resulting in a reduction in intraglomerular pressure that occurs with initiation of RAS blockade.[276]

7.8.2 Effect of sacubitril/valsartan on urinary albumin:creatinine ratio (uACR)

uACR was measured in central samples at randomization, 3, 6 and 12 months. Sacubitril/valsartan, compared with irbesartan, had no overall effect on albuminuria but, was associated with a non-significant mean reduction of 9% (95% CI -18 to 1%; P=0.08) and a trend towards lower levels of albuminuria (Table 21).

Follow-up visit	No. with value	No. with value imputed	Geometric mean (SE) Sacubitril/valsartan (n=207)	Irbesartan (n=207)	Difference in % change in geometric means (95% CI)†	P value
Randomization	414	0	34.1 (4.6)	33.9 (4.5)		
3 months	396	18	17.0 (1.0)	17.8 (1.0)	-4% (-19 to 12%)	0.58
6 months	394	20	15.6 (1.0)	18.4 (1.1)	-15% (-28 to 0%)	0.06
12 months	378	36	16.4 (1.2)	17.6 (1.3)	-6% (-23 to 14%)	0.52
Study average			**16.3 (0.6)**	**17.9 (0.7)**	**-9% (-18 to 1%)**	**0.08**

Table 22: Effect of randomization to sacubitril/valsartan on urinary albumin:creatinine ratio at 3, 6 and 12 months
†Values are percentage changes in geometric means (95% CI) for urinary albumin:creatinine ratio.

A post-hoc analysis was performed to assess whether the treatment effect of sacubitril/valsartan on albuminuria varied by subgroup (Table 23). In most subgroups examined, there was no significant difference in the effect of sacubitril/valsartan on albuminuria.

The effect of sacubitril/valsartan on albuminuria appeared to differ by underlying cause of kidney disease (P=0.01). However, even a highly significant P-value of 0.01 is not good evidence of variation in treatment on albuminuria due to the post-hoc nature of the analyses.[236,256,268] Furthermore, the result is likely to be the result of chance from multiple hypothesis testing.[238,258,268]

Subgroup	Geometric mean uACR (approx. SE) (mg/mmol) Sacubitril/valsartan (n=207)	Irbesartan (n=207)	Difference in geometric means (SE)†	Test for heterogeneity/trend χ^2 statistic	P value
Age (years)				0.59	0.44
≤60	15.2 (0.9)	17.6 (1.1)	-13% (-27 to 3%)		
>60	17.0 (0.8)	18.1 (0.8)	-6% (-17 to 7%)		
Sex				1.50	0.22
Male	16.5 (0.7)	18.8 (0.8)	-12% (-23 to -1%)		
Female	16.0 (1.1)	15.8 (1.1)	1% (-17 to 23%)		
Prior diabetes				1.09	0.30
Yes	17.5 (1.0)	20.5 (1.2)	-15% (-28 to 0%)		
No	15.6 (0.7)	16.4 (0.8)	-5% (-16 to 9%)		
Prior vascular disease				1.30	0.25
Yes	18.8 (1.4)	22.5 (1.5)	-16% (-32 to 2%)		
No	15.6 (0.7)	16.3 (0.7)	-4% (-15 to 8%)		
Systolic blood pressure (mmHg)				1.06	0.30
≤140	16.9 (1.0)	17.4 (1.0)	-3% (-17 to 15%)		
>140	16.0 (0.8)	18.3 (0.9)	-13% (-24 to 0%)		
Diastolic blood pressure (mmHg)				4.00	0.05
≤80	17.4 (0.9)	17.2 (0.9)	1% (-13 to 17%)		
>80	15.3 (0.8)	18.7 (1.0)	-18% (-29 to -5%)		
Body mass index (kg/m²)				0.21	0.64
≤30	15.6 (0.8)	17.3 (0.9)	-10% (-22 to 4%)		
>30	17.1 (0.9)	18.1 (1.0)	-6% (-19 to 10%)		
Baseline mGFR (mL/min/1.73m²)				0.04	0.85
≤30	18.2 (1.0)	19.9 (1.2)	-8% (-22 to 8%)		
>30	15.0 (0.7)	16.7 (0.8)	-10% (-21 to 3%)		
Baseline uACR (mg/mmol)				0.17	0.68
≤30	2.4 (0.2)	2.6 (0.2)	-7% (-23 to 13%)		
>30	48.5 (2.1)	54.7 (2.4)	-11% (-21 to -0%)		
Baseline 24 hour urinary sodium excretion (mg/24 hours)				1.68	0.19
≤2680	15.5 (1.1)	14.7 (1.2)	6% (-14 to 30%)		
>2680	12.7 (1.0)	14.6 (1.1)	-13% (-30 to 7%)		
Use of RAS blockade at screening				4.10	0.04
Yes	16.3 (0.7)	16.9 (0.7)	-4% (-14 to 8%)		
No	16.6 (1.5)	22.9 (2.0)	-27% (-43 to -7%)		
Cause of kidney disease				15.95	0.01
Glomerular disease	14.7 (1.0)	13.8 (1.0)	6% (-13 to 30%)		
Tubulointerstitial disease	19.9 (2.5)	17.7 (1.7)	12% (-18 to 53%)		
Diabetic kidney disease	18.9 (1.7)	19.4 (1.5)	-2% (-23 to 23%)		
Hypertensive/renovascular disease	14.7 (1.8)	21.9 (2.4)	-33% (-52 to -7%)		
Familial/hereditary nephropathies	15.3 (1.5)	20.1 (3.2)	-24% (-47 to 10%)		
Other known causes*	20.4 (4.3)	11.3 (2.4)	81% (0 to 225%)		
Unknown	16.3 (1.4)	21.6 (2.0)	-25% (-41 to -3%)		
All participants	16.3 (0.6)	17.9 (0.7)	-9% (-18 to 1%)		

Table 23: Post-hoc exploratory analysis analysing the effect of allocation to sacubitril/valsartan on study average urinary albumin:creatinine ratio in a range of subgroups

mGFR = measure glomerular filtration rate; RAS = renin angiotensin system; uACR = urinary albumin:creatinine ratio. *Includes other systemic kidney diseases.

†Values are percentage differences in geometric means (95% CIs).

7.9 Effect of sacubitril/valsartan on tertiary outcomes

7.9.1 Effect of randomization to sacubitril/valsartan on change in estimated glomerular filtration rate slopes

There was no significant difference in the rate of decline in eGFR at 12 months in those allocated sacubitril/valsartan compared with irbesartan. Mean (SE) reduction in eGFR with sacubitril/valsartan was 0.22 (0.03) mL/min/1.73m²/month and with irbesartan was 0.25 (0.03) mL/min/1.73m²/month (P=0.42; Table 24).

The rate of decline was further analysed to assess whether there was any difference between the acute slope (randomization to 3 months) and chronic slopes (3 months to 12 months). The rate of change in eGFR was fastest in the first three months following randomization with a mean (SE) decline in eGFR of 0.68 (0.12) mL/min/1.73m²/month in those allocated sacubitril/valsartan and 0.61 (0.10) mL/min/1.73m²/month in those allocated irbesartan (P=0.63; Table 23).

Estimated slopes (mL/min/1.73m²/month)	No. with value*	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	P value
Pre-specified analyses				
Randomization to 12 months	409	-0.22 (0.03)	-0.25 (0.03)	0.42
Randomization to 3 months	410	-0.68 (0.12)	-0.61 (0.10)	0.63
3 months to 12 months	409	-0.11 (0.05)	-0.16 (0.04)	0.44
Post-hoc analyses				
Randomization to 1 month	409	-2.55 (0.34)	-1.89 (0.31)	0.15
1 month to 12 months	409	-0.12 (0.04)	-0.17 (0.03)	0.33

Table 24: Effect of randomization to sacubitril/valsartan on rate of change in estimated glomerular filtration rate slopes
CKD-EPI = Chronic kidney disease Epidemiology Collaboration. eGFR = estimated glomerular filtration rate.

Additional post-hoc analysis showed most of the decline in eGFR occurred within the first month following initiation of trial treatments. The rate of decline in eGFR was faster in those allocated sacubitril/valsartan compared with irbesartan (mean [SE] decline in eGFR 2.55 [0.34] versus 1.89 [0.31] mL/min/1.73m²/month; P=0.15; Table 24). Beyond one month following randomization, the rate of change in eGFR slopes between 1 and 12 months and 3 and 12 months, was similar for both treatments (Table 24).

7.9.2 Effect of sacubitril/valsartan on blood pressure

Both systolic blood pressure and diastolic blood pressure were significantly lower in participants randomized to sacubitril/valsartan than irbesartan throughout trial follow-up (Table 25). Overall, mean systolic blood pressure was 5.4 (95% CI -7.4 to -3.4; P <0.001) mmHg lower, and mean diastolic blood pressure 2.1 (95% CI -3.3 to -1.0; P <0.001) mmHg lower in participants randomized to sacubitril/valsartan compared with irbesartan (Table 25).

The largest reduction in blood pressure was observed at 3 months. Sacubitril/valsartan reduced systolic blood pressure and diastolic blood pressure by 7.3 (95% CI -10.3 to -4.3; P<0.001) mmHg and 2.6 (95% CI -4.3 to -0.9; P=0.003) mmHg respectively, compared with irbesartan.

Follow-up visit	No. with value	No. with value imputed	Mean (SE) (mmHg) Sacubitril/valsartan (n=207)	Irbesartan (n=207)	Difference in means (95% CI)†	P value
Systolic blood pressure						
Randomization	414	0	146 (1.1)	146 (1.1)		
1 month	402	12	129 (1.1)	132 (1.1)	-3.5 (-6.5 to -0.6)	0.02
3 months	405	9	129 (1.1)	137 (1.1)	-7.3 (-10.3 to -4.3)	<0.001
6 months	396	18	128 (1.1)	135 (1.1)	-6.9 (-10.0 to -3.7)	<0.001
9 months	387	27	130 (1.2)	134 (1.2)	-4.0 (-7.3 to -0.8)	0.02
12 months	381	33	128 (2.5)	133 (2.2)	-4.4 (-10.9 to 2.1)	0.18
Study average			**129 (0.8)**	**134 (0.7)**	**-5.4 (-7.4 to -3.4)**	**<0.001**
Diastolic blood pressure						
Randomization	414	0	81 (0.8)	80 (0.8)		
1 month	402	12	73 (0.6)	74 (0.6)	-0.8 (-2.5 to 0.9)	0.37
3 months	405	9	73 (0.6)	76 (0.6)	-2.6 (-4.3 to -0.9)	0.003
6 months	396	18	72 (0.6)	75 (0.6)	-2.5 (-4.2 to -0.8)	0.005
9 months	387	27	73 (0.6)	74 (0.6)	-1.8 (-3.6 to -0.1)	0.04
12 months	381	33	72 (1.6)	75 (1.3)	-2.2 (-6.2 to 1.9)	0.29
Study average			**73 (0.5)**	**75 (0.4)**	**-2.1 (-3.3 to -1.0)**	**<0.001**

Table 25: Effect of randomization to sacubitril/valsartan on systolic and diastolic blood pressure
†Values are absolute differences in arithmetic means (95% CI).

7.9.3 Effect of sacubitril/valsartan on cardiac biomarkers

Concentrations of NT-proBNP and troponin I were similar in both treatment groups at baseline. Allocation to sacubitril/valsartan, compared with irbesartan, was associated with an 18% (95% CI -25 to -11%; P<0.001) reduction in NT-proBNP and 16% (95% CI -23% to -8%; P<0.001) reduction in troponin I concentration (Table 26).

The greatest reduction in concentrations of both biomarkers were seen at 6 months post-randomization to sacubitril/valsartan. At 6 months, allocation to sacubitril/valsartan, compared with irbesartan, was associated with 20% (95% CI -29 to -11%) lower NT-proBNP concentrations and 19% (95% CI -27 to -10%) lower troponin I concentration (Table 26).

			Geometric mean (SE) (mmHg)			
Follow-up visit	**No. with value**	**No. with value imputed**	**Sacubitril/valsartan (n=207)**	**Irbesartan (n=207)**	**Difference in geometric means (95% CI)†**	**P value**
N Terminal Pro B-type Natriuretic Peptide (ng/L)						
Randomization	414	0	254.5 (22)	250.9 (22)		
6 months	395	19	175.6 (7.2)	219.7 (8.9)	-20% (-29 to -11%)	<0.001
12 months	379	35	210.2 (11)	247.5 (12)	-15% (-26 to 2%)	0.02
Study average			**188.7 (6.0)**	**230.4 (7.3)**	**-18% (-25 to 11%)**	**<0.001**
Troponin I (ng/mL)						
Randomization	414	0	7.3 (0.5)	7.5 (0.5)		
6 months	395	19	5.4 (0.2)	6.6 (0.2)	-19% (-27 to -10%)	<0.001
12 months	379	35	6.3 (0.4)	7.1 (0.4)	-11% (-24 to 4%)	0.14
Study average			**5.7 (0.2)**	**6.8 (0.2)**	**-16% (-23 to -8%)**	**<0.001**

Table 26: Effect of randomization to sacubitril/valsartan on cardiac biomarkers
†Values are percentage changes in geometric means (95% CI).

Additional post-hoc analyses showed that the effect of sacubitril/valsartan on NT-proBNP did not differ by any particular baseline characteristic (Table 27). However, the effect of troponin I appeared to be differ significantly with BMI. Sacubitril/valsartan, compared with irbesartan, appeared to have a significantly larger effect on those with a BMI greater than 30, resulting in a 28% (-37 to -18%; P<0.001) reduction in troponin I concentrations (Table 28).

Subgroup	Geometric mean NT-proBNP (approx. SE) (ng/L) Sacubitril/valsartan (n=207)	Irbesartan (n=207)	Difference in geometric means (SE)†	Test for heterogeneity/trend χ^2 statistic	P value
Age (years)				1.22	0.27
≤60	160.9 (8.4)	208.2 (12)	-23% (-33 to -10%)		
>60	208.3 (8.3)	242.9 (9.5)	-14% (-23 to -4%)		
Sex				0.95	0.33
Male	186.2 (7.0)	221.5 (8.1)	-16% (-24 to -7%)		
Female	194.9 (12)	255.6 (16)	-24% (-35 to -10%)		
Prior diabetes				1.98	0.16
Yes	212.8 (11)	240.0 (12)	-11% (-23 to 2%)		
No	174.7 (7.1)	224.2 (9.1)	-22% (-30 to -13%)		
Prior vascular disease				1.40	0.24
Yes	212.0 (14)	235.6 (14)	-10% (-24 to 7%)		
No	181.7 (6.7)	228.3 (8.7)	-20% (-28 to -12%)		
Systolic blood pressure (mmHg)				0.00	0.97
≤140	188.0 (9.8)	229.3 (11)	-18% (-29 to -6%)		
>140	189.1 (7.6)	231.3 (9.6)	-18% (-27 to -8%)		
Diastolic blood pressure (mmHg)				1.49	0.22
≤80	204.9 (9.4)	236.2 (10)	-13% (-23 to -2%)		
>80	174.4 (7.8)	224.5 (10)	-22% (-31 to -12%)		
Body mass index (kg/m²)				0.46	0.50
≤30	179.8 (7.7)	224.7 (9.8)	-20% (-29 to -10%)		
>30	192.0 (9.0)	225.8 (10)	-15% (-25 to -3%)		
Baseline mGFR (mL/min/1.73m²)				0.25	0.62
≤30	217.7 (11)	260.7 (13)	-17% (-27 to -4%)		
>30	169.5 (7.1)	212.4 (8.6)	-20% (-29 to -11%)		
Baseline uACR (mg/mmol)				0.25	0.62
≤30	201.2 (11)	239.3 (13)	-16% (-28 to -2%)		
>30	181.4 (7.1)	226.2 (8.9)	-20% (-28 to -11%)		
Baseline 24 hour urinary sodium excretion (mg/24 hours)				1.44	0.23
≤2680	206.0 (13)	241.6 (17)	-15% (-29 to 3%)		
>2680	162.9 (11)	224.5 (15)	-27% (-40 to -13%)		
Use of RAS blockade at screening				0.23	0.63
Yes	185.7 (6.5)	223.6 (7.9)	-17% (-25 to -8%)		
No	204.4 (16)	260.3 (19)	-21% (-36 to -3%)		
Cause of kidney disease				6.62	0.36
Glomerular disease	170.9 (10)	221.3 (14)	-23% (-35 to -8%)		
Tubulointerstitial disease	185.8 (20)	225.5 (18)	-18% (-37 to 7%)		
Diabetic kidney disease	228.5 (17)	231.4 (15)	-1% (-19 to 20%)		
Hypertensive/renovascular disease	175.3 (18)	227.7 (21)	-23% (-42 to 1%)		
Familial/hereditary nephropathies	174.1 (15)	249.8 (33)	-30% (-49 to -5%)		
Other known causes§	203.8 (37)	183.2 (33)	11% (-33 to 84%)		
Unknown	201.7 (15)	252.9 (20)	-20% (-35 to -1%)		
All participants	188.7 (6.0)	230.4 (7.3)	-18% (-25 to -11%)		

Table 27: Post-hoc assessment of the effect of sacubitril/valsartan on NT-proBNP
mGFR = measure glomerular filtration rate; NT-proBNP = N-Terminal pro B-type Natriuretic Peptide; RAS = renin angiotensin system; uACR=urinary albumin:creatinine ratio. †Values are percentage differences in geometric means (95% CI). §Includes other systemic kidney diseases.

Subgroup	Geometric mean troponin I (approx. SE) (ng/L) Sacubitril/valsartan (n=207)	Irbesartan (n=207)	Difference in geometric means (SE)†	Test for heterogeneity/trend χ^2 statistic	P value
Age (years)				0.52	0.47
≤60	5.3 (0.3)	6.0 (0.3)	-12% (-24 to 2%)		
>60	6.0 (0.2)	7.3 (0.3)	-18% (-26 to -8%)		
Sex				0.29	0.59
Male	5.9 (0.2)	7.1 (0.3)	-17% (-25 to -8%)		
Female	5.3 (0.3)	6.0 (0.4)	-13% (-26 to 4%)		
Prior diabetes				0.00	0.98
Yes	5.9 (0.3)	7.1 (0.4)	-16% (-27 to -3%)		
No	5.6 (0.2)	6.6 (0.3)	-16% (-25 to -6%)		
Prior vascular disease				0.20	0.65
Yes	6.7 (0.4)	8.2 (0.5)	-18% (-30 to -3%)		
No	5.4 (0.2)	6.3 (0.2)	-14% (-22 to -5%)		
Systolic blood pressure (mmHg)				0.21	0.65
≤140	5.8 (0.3)	6.7 (0.3)	-14% (-25 to -1%)		
>140	5.7 (0.2)	6.9 (0.3)	-17% (-26 to -7%)		
Diastolic blood pressure (mmHg)				0.17	0.68
≤80	6.0 (0.3)	7.0 (0.3)	-14% (-24 to -3%)		
>80	5.5 (0.2)	6.6 (0.3)	-17% (-27 to -6%)		
Body mass index (kg/m²)				12.79	<0.001
≤30	6.1 (0.3)	6.1 (0.3)	-1% (-12 to 11%)		
>30	5.4 (0.3)	7.5 (0.3)	-28% (-37 to -18%)		
Baseline mGFR (mL/min/1.73m²)				0.90	0.34
≤30	5.9 (0.3)	7.4 (0.4)	-20% (-31 to -9%)		
>30	5.6 (0.2)	6.4 (0.3)	-13% (-23 to -3%)		
Baseline uACR (mg/mmol)				0.00	0.97
≤30	5.5 (0.3)	6.5 (0.4)	-16% (-28 to -2%)		
>30	5.9 (0.2)	7.0 (0.3)	-16% (-24 to -6%)		
Baseline 24 hour urinary sodium excretion (mg/24 hours)				0.02	0.89
≤2680	5.8 (0.4)	6.9 (0.5)	-16% (-30 to 1%)		
>2680	6.2 (0.4)	7.3 (0.5)	-14% (-29 to 3%)		
Use of RAS blockade at screening				0.00	0.95
Yes	5.7 (0.2)	6.8 (0.2)	-16% (-23 to -7%)		
No	5.9 (0.5)	7.0 (0.5)	-16% (-32 to 3%)		
Cause of kidney disease				2.11	0.91
Glomerular disease	5.3 (0.3)	6.4 (0.4)	-18% (-30 to -3%)		
Tubulointerstitial disease	6.0 (0.6)	7.0 (0.6)	-14% (-34 to 12%)		
Diabetic kidney disease	6.0 (0.5)	7.2 (0.5)	-16% (-31 to 2%)		
Hypertensive/renovascular disease	6.2 (0.6)	6.2 (0.6)	-1% (-25 to 31%)		
Familial/hereditary nephropathies	6.2 (0.5)	8.3 (1.1)	-25% (-45 to 1%)		
Other known causes§	6.0 (1.1)	7.2 (1.3)	-17% (-50 to 37%)		
Unknown	5.4 (0.4)	6.5 (0.5)	-17% (-33 to 2%)		
All participants	5.7 (0.2)	6.8 (0.2)	-16% (-23 to -8%)		

Table 28: Post-hoc assessment of the effect of sacubitril/valsartan on troponin I
mGFR = measure glomerular filtration rate; RAS = renin angiotensin system; uACR = urinary albumin:creatinine ratio. §Includes other systemic kidney diseases. †Values are percentage differences in geometric means (95% CI).

7.9.4 Pharmacokinetic analyses

Data from 87 participants who had taken their last dose of sacubitril/valsartan between 10 and 16 hours previously were used to assess the effects of determinants of drug metabolism and drug levels. Median (IQR) concentrations of each metabolite were: sacubitril 2 (1-5) ng/mL, sacubitrilat 11700 (8700-15900) ng/mL and valsartan 975 (522-1910) ng/mL.

Concentrations of sacubitrilat (the active form of sacubitril) increased significantly with decreasing kidney function. Each 10 mL/min/1.73m^2 reduction in mGFR was associated with a 1485 (95% CI 572 to 2397) ng/mL higher sacubitrilat concentration (P=0.002; Table 28). The median (IQR) concentration of sacubitrilat in participants with an mGFR of 30 mL/min/1.73m^2 or below was 16,700 (10500-19400) ng/mL compared with 11100 (8170-15300) ng/mL in participants with an eGFR above 30 mL/min/1.73m^2.

	Sacubitril		Sacubitrilat		Valsartan	
Characteristic	**Percentage change (95% CI)**	**P value**	**Absolute change in ng/mL (95% CI)**	**P value**	**Percentage change (95% CI)**	**P value**
Age, per decade higher	22% (1 to 48%)	0.04	889 (-30 to 1808)	0.06	14% (-2 to 33%)	0.09
Race*		0.78		0.71		0.22
Black	-60% (-95 to 252%)		-4856 (-15440 to 5729)		-63% (-94 to 112%)	
Other	-44% (-89 to 175%)		539 (-7208 to 8286)		5% (-71 to 276%)	
South Asian	7% (-71 to 297%)		1947 (-3684 to 7578)		-59% (-84 to 5%)	
Sex†	-25% (-67 to 71%)	0.48	-4413 (-8444 to -381)	0.03	-37% (-67 to 23%)	0.18
BSA, per 0.1 m² higher	-32% (-64 to 31%)	0.25	-3327 (-6500 to -154)	0.04	-23% (-54 to 31%)	0.34
Weight, per 5 kg higher	20% (-20 to 78%)	0.37	1915 (-25 to 3854)	0.05	13% (-18 to 56%)	0.44
mGFR (unadjusted for BSA), per 10 mL/min/1.73m² lower	-17% (-32 to 1%)	0.06	1485 (572 to 2397)	0.002	-3% (-17 to 13%)	0.65
Log ACR, per 5-fold increase	-7% (-24 to 13%)	0.44	-622 (-1590 to 346)	0.21	-14% (-27 to 1%)	0.07

Table 29: Associations between baseline characteristics and sacubitril/valsartan metabolite values at the 3 month visit

ACR = albumin:creatinine ratio; BSA = body surface area; mGFR = measured glomerular filtration rate. Models adjusted for all characteristics shown in table and additionally for time since last dose. *White ethnicity used as reference category. Race was not prescriptive for inclusion in the models. †Males used as a reference category.

7.10 Safety outcomes with sacubitril/valsartan

7.10.1 Effect of sacubitril/valsartan on serious adverse events (SAEs)

There were two deaths during the trial, one due to pulmonary embolism in a participant randomized to sacubitril/valsartan and the other due to sepsis in a participant randomized to irbesartan. Neither of these deaths were believed to be related to study treatment (Table 29).

One case of angioedema occurred in a participant randomized to sacubitril/valsartan (Table 29). However, this was an expected adverse reaction with sacubitril/valsartan and the participant did not seek medical attention. Only one serious adverse event of significant hypotension was reported in each treatment group (Table 29).

Requirement for temporary dialysis was similar in both treatment groups (2 participants randomized to sacubitril/valsartan and 3 participants randomized to irbesartan: Table 29). Only two participants progressed to ESKD requiring long-term dialysis; both were randomized to irbesartan. There was no numerical excess in numbers of cases of any other non-fatal serious adverse events. Overall rates of all serious adverse events did not differ significantly between those allocated sacubitril/valsartan, compared with irbesartan (61/207 [29.5%] versus 59/207 (28.5%) respectively; rate ratio [RR] 1.07 [95% CI 0.75-1.53]; P=0.70) (Table 29A&B).

	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	Rate ratio (95% CI)	P value
Fatal SAEs				
Cardiovascular causes				
Coronary	0 (0.0%)	0 (0.0%)		
Other cardiac	0 (0.0%)	0 (0.0%)		
Other vascular	0 (0.0%)	0 (0.0%)		
Subtotal: Any cardiovascular	0 (0.0%)	0 (0.0%)		
Non-cardiovascular causes				
Cancer	0 (0.0%)	0 (0.0%)		
Infection	0 (0.0%)	1 (0.5%)		
Respiratory*	1 (0.5%)	0 (0.0%)		
Hepatic*	0 (0.0%)	0 (0.0%)		
Other medical	0 (0.0%)	0 (0.0%)		
Non-medical	0 (0.0%)	0 (0.0%)		
Subtotal: Any non-cardiovascular	1 (0.5%)	1 (0.5%)		
Subtotal: Uncategorized/unknown	0 (0.0%)	0 (0.0%)		
Total: Any fatal SAE	1 (0.5%)	1 (0.5%)		

Table 30A: Effect of randomization to sacubitril/valsartan on serious adverse events

	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	Rate ratio (95% CI)	P value
Non-fatal SAEs				
Angioedema	1 (0.5%)	0 (0.0%)		
Hypotension	1 (0.5%)	1 (0.5%)		
Dialysis	2 (1.0%)	3 (1.4%)		
Other SAE (by MedDRA* System, Organ, Class [SOC] category)				
Respiratory, thoracic and mediastinal Disorders	6 (2.9%)	6 (2.9%)		
Infection and infestations	16 (7.7%)	15 (7.2%)		
Blood and lymphatics system	2 (1.0%)	2 (1.0%)		
Cardiac disorders	6 (2.9%)	5 (2.4%)		
Ear disorders	0 (0.0%)	0 (0.0%)		
Endocrine disorders	2 (1.0%)	0 (0.0%)		
Eye disorders	2 (1.0%)	1 (0.5%)		
Gastrointestinal disorders	5 (2.4%)	6 (2.9%)		
Hepatobiliary disorders				
Bile duct and gallbladder disorders	1 (0.5%)	1 (0.5%)		
Liver/other hepatobiliary	0 (0.0%)	0 (0.0%)		
Immune system disorders	0 (0.0%)	0 (0.0%)		
Metabolism and nutrition disorders				
Diabetes/glucose	3 (1.4%)	1 (0.5%)		
Other metabolism/nutrition	7 (3.4%)	6 (2.9%)		
Musculoskeletal and connective tissue Disorders	3 (1.4%)	0 (0.0%)		
Cancer	4 (1.9%)	5 (2.4%)		
Neoplasms benign, malignant and unspecified (including cysts and polyps)	2 (1.0%)	3 (1.4%)		
Nervous system disorders				
Stroke or TIA	1 (0.5%)	1 (0.5%)		
Other neurological	2 (1.0%)	2 (1.0%)		
Psychiatric disorders	0 (0.0%)	0 (0.0%)		
Renal and urinary disorders	10 (4.8%)	5 (2.4%)		
Skin and subcutaneous tissue disorders (excluding angioedema)	1 (0.5%)	0 (0.0%)		
Vascular disorders (excluding hypotension)	0 (0.0%)	1 (0.5%)		
Other medical	30 (14.5%)	29 (14.0%)		
Investigations	8 (3.9%)	13 (6.3%)		
Surgical and medical procedures (excluding dialysis)	18 (8.7%)	14 (6.8%)		
Miscellaneous medical**	13 (6.3%)	8 (3.9%)		
Non-medical (including trauma)	7 (3.4%)	5 (2.4%)		
Total: Any non-fatal SAE	**61 (29.5%)**	**59 (28.5%)**	**1.07 (0.75-1.53)**	**0.70**
Total: Any SAE	**61 (29.5%)**	**59 (28.5%)**	**1.07 (0.75-1.53)**	**0.70**

Table 31B: Effect of randomization to sacubitril/valsartan on serious adverse events

*MedRA = Medical dictionary for Regulatory Activities; SAE = serious adverse event; TIA=Transient ischaemic attack. *Excluding cancer and infection. **Made up of System Organ Class categories: Congenital, familial and genetic disorders, General disorders and administration site conditions, and Pregnancy, puerperium and perinatal conditions.

7.10.2 Effect of sacubitril/valsartan on non-serious adverse reactions (NSARs)

Non-serious adverse reactions (reported by trial participants or study nurses as being related to study treatments) of hypotension, hyperkalaemia and acute kidney injury were considered to be adverse events of special interest. These events might result in withdrawal of the study treatment and have significant impact on kidney function in people with CKD.

Rates of non-serious adverse reactions of hypotension with sacubitril/valsartan were more than double that with irbesartan (17/207 [8.2%] versus 7/207 [3.4%] respectively; RR 2.36 [95% CI 1.06-5.26]; P=0.04; Table 30). There were more cases of hyperkalaemia with sacubitril/valsartan compared with irbesartan (6/207 [2.9%] versus 1/207 [0.5%] respectively; RR 4.23 [95% CI 0.96-18.61]; P=0.06). Rates of AKI were similar between sacubitril/valsartan and irbesartan (3/207 [1.4%] versus 6/207 [2.9%] respectively; RR 0.51 [95% CI 0.14-1.90]; P=0.32).

Cases of gastrointestinal disorders (for example nausea, vomiting, diarrhoea) were more frequently reported in participants allocated sacubitril/valsartan than irbesartan (18/207 [8.7%] versus 10/207 [4.8%] respectively), as were skin and subcutaneous tissue disorders including pruritus (18/207 [8.7%] versus 6/207 [2.9%] respectively; Table 30).

Overall, rates of non-serious adverse reactions were similar between sacubitril/valsartan and irbesartan (76/207 [36.7%] versus 58/207 [28.0%] respectively; RR 1.35 [95% CI 0.96-1.90]; P=0.08; Table 30A&B).

	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	Rate ratio (95% CI)	P value
Hypotension	17 (8.2%)	7 (3.4%)	2.36 (1.06-5.26)	0.04
Hyperkalaemia	6 (2.9%)	1 (0.5%)	4.23 (0.96-18.61)	0.06
Acute kidney injury	3 (1.4%)	6 (2.9%)	0.51 (0.14-1.90)	0.32

Table 32A: Effect of sacubitril/valsartan on non-serious adverse reactions

	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	Rate ratio (95% CI)	P value
Other NSAR (by MedDRA System, Organ, Class [SOC] category)				
Respiratory, thoracic and mediastinal disorders	4 (1.9%)	4 (1.9%)		
Infection and infestations	2 (1.0%)	1 (0.5%)		
Blood and lymphatics system	1 (0.5%)	0 (0.0%)		
Cardiac disorders	0 (0.0%)	2 (1.0%)		
Ear disorders	1 (0.5%)	2 (1.0%)		
Endocrine disorders	0 (0.0%)	0 (0.0%)		
Eye disorders	0 (0.0%)	1 (0.5%)		
Gastrointestinal disorders	18 (8.7%)	10 (4.8%)		
Hepatobiliary disorders				
Bile duct and gallbladder Disorders	0 (0.0%)	0 (0.0%)		
Liver/other hepatobiliary	0 (0.0%)	0 (0.0%)		
Immune system disorders	0 (0.0%)	0 (0.0%)		
Metabolism and nutrition disorders (excluding hyperkalaemia)				
Diabetes/glucose	0 (0.0%)	0 (0.0%)		
Other metabolism/nutrition	3 (1.4%)	1 (0.5%)		
Musculoskeletal and connective tissue disorders	6 (2.9%)	5 (2.4%)		
Cancer	0 (0.0%)	0 (0.0%)		
Neoplasms benign, malignant and unspecified (incl. cysts and polyps)	0 (0.0%)	0 (0.0%)		
Nervous system disorders				
Stroke or TIA	0 (0.0%)	0 (0.0%)		
Other neurological	20 (9.7%)	18 (8.7%)		
Psychiatric disorders	0 (0.0%)	1 (0.5%)		
Renal and urinary disorders (excluding acute kidney injury)	2 (1.0%)	2 (1.0%)		
Reproductive system and breast disorders	2 (1.0%)	3 (1.4%)		
Skin and subcutaneous tissue disorders (excluding angioedema)	18 (8.7%)	6 (2.9%)		
Vascular disorders (excluding hypotension)	1 (0.5%)	0 (0.0%)		
Other medical	6 (2.9%)	7 (3.4%)		
Investigations	3 (1.4%)	1 (0.5%)		
Surgical and medical procedures	0 (0.0%)	0 (0.0%)		
Miscellaneous medical**	8 (3.90%)	12 (5.8%)		
Non-medical (including trauma)	0 (0.0%)	0 (0.0%)		
Total: Any non-serious adverse reaction*	76 (36.7%)	58 (28.0%)	1.35 (0.96-1.90)	0.08

Table 33B: Effect of sacubitril/valsartan on non-serious adverse reactions
NSAR=non-serious adverse reaction. TIA=Transient ischaemic attack. *Excluding angioedema. **Made up of SOC categories: Congenital, familial and genetic disorders, General disorders and administration site conditions, and Pregnancy, puerperium and perinatal conditions.

7.10.3 Effect of sacubitril/valsartan on biochemical safety parameters

7.10.3.1 *Renal safety data*

Overall, rates of hyperkalaemia (potassium result of 5.5 mmol/L or higher) were not significantly different; 32% (66/207) participants allocated sacubitril/valsartan compared with 24% (50/207) allocated irbesartan, experienced hyperkalaemia (P=0.10; Table 31).

Moderate hyperkalaemia (potassium between 6.0 and 6.5 mmol/L) occurred in 10% (20/207) participants allocated sacubitril/valsartan compared with 3% (7/207) allocated irbesartan. There was no difference in rates of severe hyperkalaemia (potassium 6.5 mmol/L or higher) between sacubitril/valsartan vs irbesartan (2/207 [1%] with versus 5/207 [2%] respectively; Table 30).

Similarly, there was no difference in reduction in CKD-EPI eGFR of 25% or greater compared with eGFR at randomization. This degree of decline in eGFR occurred in 34% (71/207) of participants allocated sacubitril/valsartan compared with 32% (67/207) allocated irbesartan (P=0.75) (Table 31).

Renal outcome*	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	P-value
Potassium (mmol/L)			
≥5.5 to <6.0	44 (21%)	38 (18%)	
≥6.0 to <6.5	20 (10%)	7 (3%)	
≥6.5	2 (1%)	5 (2%)	
Total: Any hyperkalaemia	66 (32%)	50 (24%)	0.10
CKD-EPI eGFR			
≥25% reduction in CKD-EPI eGFR	71 (34%)	67 (32%)	0.75

Table 34: Effect of sacubitril/valsartan on renal safety outcomes
CKD-EPI = Chronic kidney disease Epidemiology Collaboration; eGFR = estimated glomerular filtration rate. *Based on local laboratory measurements.

7.10.3.2 *Liver safety data*

No cases of significant liver enzyme derangement were observed with either sacubitril/valsartan or irbesartan (Table 32).

Liver outcome*	Sacubitril/valsartan (n=207)	Irbesartan (n=207)
ALT/AST >10x ULN	0 (0%)	0 (0%)
Consecutive ALT/AST >3x ULN	0 (0%)	0 (0%)
ALT/AST >3x ULN and bilirubin ≥2x ULN	0 (0%)	0 (0%)

Table 35: Effect of sacubitril/valsartan on liver safety outcomes
ALT = alanine transaminase; AST = aspartate transaminase; ULN = upper limit of normal.
*Based on local laboratory measurements

Two participants developed abnormalities in liver enzymes which did not meet the criteria for liver enzyme derangement outlined in Table 32. One participant had an isolated rise in ALT 6x ULN which was believed to be due to acute decompensated heart failure. The liver enzymes normalized without any specific treatment despite continuing the study treatments.

The second participant developed an ALT rise of 2.7x ULN and an associated bilirubin rise of more than 4x ULN due to cholecystitis and biliary sepsis. Study treatments were discontinued as the participant simultaneously developed significant AKI. Neither of the biochemical complications were believed to be related to study treatment.

8 Discussion

The UK HARP-III trial is the first randomized trial to examine the effects of ARNI with sacubitril/valsartan on kidney function in patients with advanced CKD. Trials of sacubitril/valsartan in patients with heart failure and hypertension have excluded patients with advanced CKD (particularly those with an eGFR less than 30 mL/min/1.73m^2).[190,192,194,196]

The UK HARP-III trial demonstrated twelve months of treatment with sacubitril/valsartan had similar effects on kidney function to irbesartan in patients with CKD. In contrast to patients with heart failure, no significant effect on albuminuria was observed. Sacubitril/valsartan substantially reduced blood pressure, cardiac biomarker concentrations and, had similar tolerability without any major safety concerns to irbesartan.

8.1 Recruitment successes and challenges

The pre-screening method (pre-screening potentially eligible participants and telephoning them about a week later) used for recruiting patients into UK HARP-III proved very successful. Screening rates were numerically much higher (21.9 patients per million population per year) at sites primarily using pre-screening methods compared with sites primarily using traditional recruitment methods of recruitment (14.1 patients per million population per year). This method of recruitment has been extremely successful in other large randomized trials in cardiovascular disease and diabetes, with telephone reminders following initial invitation increasing recruitment by 6% (95% CI 3% to 9%).[273,277-280] Trials using 'traditional' methods of recruitment have reported much slower rates of recruitment.[278,281]

Successful trial recruitment is pivotal to the outcome of a trial. Poor recruitment can substantially increase trial costs or cause premature trial discontinuation resulting in an underpowered trial which could incorrectly show a statistically non-significant result and risk the trial treatment or intervention being inappropriately withdrawn.[282] If trial follow-up were lengthened to account for poor or slow recruitment, participants could be exposed to potentially hazardous or ineffective treatments for longer

durations or conversely result in substantial delays in demonstrating any clinical benefit.[280]

There are several reasons that contributed to the success of the recruitment method used in UK HARP-III. CCO staff made regular (weekly) contact with study nurses to enquire about recruitment progress at local sites. CCO staff collected and recorded information on the numbers of invitations sent, numbers of patients telephoned and those agreeing to participate each week. Sites recruiting more slowly were contacted to discuss potential reasons for this. A monthly newsletter was produced containing a 'league' table of recruitment performance by site, allowing local study staff to compare their own recruitment progress with other sites. Enhanced communication with sites and provision of site-specific feedback has been shown to increase numbers of participants in clinical trials and reduce the duration of recruitment,[280] and such approaches have proven to be successful in other trials.[278]

The UK HARP-III trial eligibility criteria were broad and simple to encourage the recruitment of a wide range of participants. This strategy ensures trial results are widely generalizable and provide more reliable information regarding the safety and efficacy of treatments such as sacubitril/valsartan.[282]

Renal units from across England, Scotland and Wales were invited to participate in the trial ensuring that the diverse range of CKD patients managed across the UK were eligible to participate, with the trial conducted within the usual clinical setting. Trial populations do not necessarily need to be 'representative' of the population from which they are recruited for trial results to be widely generalizable.[282] Adequate randomization and balance of potential confounders between the treatment arms is essential to enable the proportional effects of the treatment on various outcomes to studied and applied to the wider population of interest.[282]

Baseline characteristics in UK HARP-III were well-balanced between both treatment groups. Participants were mainly white and male. Cardiovascular disease was present in about 10% and diabetes mellitus in 40% of participants. The aetiology of CKD amongst participants was similar to that seen in UK renal units.[283] Blood pressure was slightly higher (146/81) than expected for patients with CKD managed in secondary care. However, pre-study RAS inhibition (taken by 82% of participants) was stopped at screening resulting in a slight rise in blood pressure during the pre-randomization run-in prior to initiation of randomized study treatment. The majority of participants had CKD stage 3 or 4 and over two-thirds had macroalbuminuria,

representing a population at risk of progression of CKD. This was verified by assessment of risk of progression to ESRD using an established risk equation which showed two-thirds of UK HARP-III participants had a 5 year risk of at least 10%, and their overall lifetime risk was probably much higher, although current equations do not calculate such risk.[275]

The number of female and elderly (aged 70 years or older) participants recruited to UK HARP-III was lower than males and younger counterparts respectively which has been seen in many cancer and cardiovascular disease trials.[56,284-286] However, it is not clear whether such individuals were not invited to participate in UK HARP-III by local centres or whether they were more likely to decline to participate compared with male counterparts as this information was not collected centrally.

The numbers of participants recruited from ethnic minority groups were low although similar to those recruited in trials in other disease areas. Several UK HARP-III participating centres were based in areas with large populations of ethnic minority groups and in these centres, translators were readily available for routine medical appointments and study visits to prevent language barriers hindering trial participation. The participant information sheets and study documentation (such as study posters, consent forms, study website) were only published in the English language therefore, non-English speaking patients may have been disadvantaged but English language was not an entry requirement for eligibility in UK HARP-III.

Low rates of participation amongst ethnic minorities have been reported in other large randomized trials and,[64,287,288] in trials of sacubitril/valsartan.[190,192,194] Underrepresentation of individuals from ethnic minorities could have potential implications for assessment of effects of genetic variability in metabolism and treatment tolerance.[289,290] Factors that have been described as potential contributors to underrepresentation of ethnic minorities in medical research include: increasing participant age; sex; fear and mistrust in medical research/researchers; lack of awareness about medical research; language barriers; socioeconomic factors; concerns regarding drug side effects and; cultural and religious beliefs.[285,291-293]

In future large clinical trials, to ensure recruitment of people from ethnic minority groups, clinical trials should aim to recruit participants from native countries.

8.2 Tolerability and compliance with sacubitril/valsartan

Sacubitril/valsartan had similar tolerability and compliance to irbesartan throughout the trial. The use of a single-blind placebo run-in phase is a recognised method for improving compliance by identifying those participants that may be unwilling or unable to comply with study treatments and/or follow-up for the study duration. If such patients were randomized it is likely that they would discontinue study treatments or attendance at follow-up visits post-randomization, which could have a significant impact on the trial's statistical power.[245,246]

The trial steering committee monitored the reasons for drop out during run-in. The majority of participants withdrawn from run-in had ineligible laboratory results on samples taken at screening. In view of this, the Trial Steering Committee (TSC) recommended a change in the study protocol allowing results that did not fulfil the trial eligibility criteria to be repeated once more for confirmation of eligibility. This reduced drop-out from run-in due to ineligible laboratory results.

Drop-out at randomization was higher than expected. On cessation of prior RAS blockade at screening, some participants were initiated on additional blood pressure lowering medication causing blood pressure to fall below the level for eligibility at randomization (greater than 130/80). The TSC recommended the lower limit for systolic blood pressure be reduced to less than 110 mmHg (or below 130 mmHg if the patient had symptomatic hypotension). These changes to the eligibility criteria improved the efficiency of the trial without adversely affecting the reliability of the trial results or compromising patient safety.

Compliance with study follow-up visits was excellent throughout the trial, with very few missed follow-up visits. As per intention to treat analyses, all patients were asked to continue to attend for study follow-up (either in person, by telephone or by use of their medical records) even if they stopped study treatments. To facilitate this, I telephoned all study nurses when informed of participants with a change in their study treatment or follow-up method and/or when reviewing completed study follow-up forms. A log of all such participants was maintained and I regularly contacted LRCs to review compliance of affected individuals and encourage maximum compliance wherever possible. Participants indicating that they no longer wished to continue with study follow-up visits were contacted to reiterate the importance of ongoing trial follow-up despite stopping study treatments.

The initial trial power calculation assumed randomization of 360 participants would provide 80% power (at 2p=0.05) to detect a clinically meaningful difference in mGFR of 3 mL/min/1.73m^2, even if 10% of participants discontinued study treatment after randomization. During the trial, post-hoc data from the PARAMOUNT trial suggested it may take up to 9 months for the effects of sacubitril/valsartan on renal function to emerge.[294] The TSC therefore recommended increasing trial follow-up to 12 months. The revised power calculation took into account any change in compliance with study treatment over an extended period of follow-up and so randomization of 400 participants provided similar power even if 15% of participants discontinued study treatment.

Throughout the trial, rates of discontinuation of study treatments were similar at each follow-up time-point. At 12 months, about 80% of participants were still taking some study treatment and the proportion taking full dose sacubitril/valsartan or irbesartan dropped by similar amounts in each treatment group. Reasons for discontinuing sacubitril/valsartan were similar to other trials.[190,194,195]

In PARADIGM-HF, overall rates of discontinuation of sacubitril/valsartan were significantly lower than enalapril (746/4187 [17.8%] versus 833/4212 [19.8%] respectively; HR 0.89 [95% CI 0.80-0.98]; P=0.016).[202] In participants with CKD the rates of study treatment discontinuation were similar in both treatment groups (324/2745 [24%] sacubitril/valsartan versus 355/5654 [25%] enalapril; HR 0.97 [95% CI 0.84-1.13]; P=0.72) and, did not differ significantly from those without CKD (P for interaction = 0.18).[202] Renal reasons accounted for fewer discontinuations of sacubitril/valsartan than enalapril amongst the overall trial population (27/4189 [0.9%] versus 59/4212 [1.4%] respectively; HR 0.49 [95% CI 0.31-0.76]; P=0.002).

In UK HARP-III, there were numerically (though not statistically significantly) more cases of hypotension resulting in a reduction in the dose of sacubitril/valsartan. This was not surprising given the effect of the drug on blood pressure and hypotension did not produce any excess in discontinuations of sacubitril/valsartan, compared with irbesartan, as in other trials.[194]

8.3 Effects of sacubitril/valsartan on renal function

At 12 months, sacubitril/valsartan had a similar effect to irbesartan on mGFR. The effect of sacubitril/valsartan on mGFR was not modified by any particular baseline characteristic (including: age; sex; presence of diabetes; previous vascular disease; severity of renal impairment; albuminuria; urinary sodium excretion at baseline; prior use of RAS blockade; BMI; blood pressure or; cause of underlying renal disease), suggesting the drug has similar effects across the broad range of patients with CKD studied in UK HARP-III.

Animal studies of neprilysin inhibition with omapatrilat (vasopeptidase inhibition) showed similar effects on renal function to those in UK HARP-III. In a variety of animal models of renal disease, treatment with omapatrilat was not associated with significant differences in GFR compared with animals treated with ACE inhibition alone.[158,159] In contrast, histology specimens from these animals showed combined NEP/RAS inhibition, was associated with substantial reductions in glomerulosclerosis and tubulointerstitial fibrosis compared with isolated RAS inhibition, suggesting greater renoprotection with the addition of NEP inhibition.[138,158,159,161]

Animal studies that compared ARBs combined with NEP inhibition (mimicking ARNIs) with isolated RAS inhibition (with an ARB), reported similar GFR and histological findings to studies with vasopeptidase inhibitors.[199,295] Although animal models are poorly predictive of drug efficacy in humans, these data were encouraging and provided a rationale for the potential use of NEP inhibition in people with CKD to preserve kidney function.[162,163]

The animal data suggest that the duration of the UK HARP-III trial may have been too short to detect a clinically meaningful effect on kidney function. Sacubitril/valsartan may also need to be given for a much longer duration to allow the histological findings seen in animal studies to translate into biochemical and clinical efficacy in humans. Trials examining the effects of isolated RAS (compared with placebo and/or other antihypertensive treatment) on ESKD in people with diabetic nephropathy, showed it may take 15-18 months for the effects on this outcome to emerge.[41,42] The effects of isolated RAS inhibition on doubling in serum creatinine were observed earlier, at around 12 months.[41,42]

The results from the PARADIGM-HF trial suggested sacubitril/valsartan may have smaller effects on renal function than those in the PARAMOUNT trial and, that the effects may take longer to emerge.[200,202] In patients with CKD, combined NEP/RAS inhibition may need to be initiated at much earlier stages of kidney disease, prior to the development of substantial renal fibrosis, nephron loss and hyperfiltration causing albuminuria, to have an influential effect on kidney function. The UK HARP-III trial was not intended (and the trial duration was too short) to determine any longer-term effects of ARNIs on renal outcomes such as progression to ESKD in people with CKD.

The renal function results from the UK HARP-III trial contrast to those in trials of heart failure populations. In PARAMOUNT, patients with HFpEF randomized to sacubitril/valsartan, had a slower decline in eGFR following 36 weeks treatment compared with valsartan (1.5±13.1 versus 5.2±11.4 mL/min/1.73m^2 respectively; P=0.008).[200] However, PARAMOUNT participants had a higher eGFR (mean 65.5 [SD 20.4] mL/min/1.73m^2) compared with UK HARP-III participants and only 42% had CKD (defined as an eGFR less than 60 mL/min/1.73m^2) at baseline.[195,200]

In PARADIGM-HF, allocation to sacubitril/valsartan resulted in a slower rate of decline in eGFR between screening and the end of follow-up, compared with enalapril (1.61 [95% CI -1.77 to -1.44] versus 2.04 [95% CI -2.21 to -1.88] mL/min/1.73m^2/year respectively; P<0.001).[202] The effect on eGFR was similar in patients with and without CKD at baseline (P for interaction = 0.54).[202] Post-hoc analyses suggested sacubitril/valsartan may have a greater effect on slowing the rate of decline in renal function, in people with diabetes compared to those without diabetes.[203]

In UK HARP-III there was no heterogeneity in the treatment effect by diabetes status. The lack of effect of sacubitril/valsartan on GFR seen in UK HARP-III may result from differences in the mechanisms of progression of kidney disease in people with heart failure compared with those with more advanced and/or proteinuric CKD. Neprilysin inhibition raises circulating levels of natriuretic peptides (NPs) which causes systemic vasodilatation and reduces systemic blood pressure and renal blood flow.[158,161]

In people with heart failure, cardiac function is probably a more important determinant of kidney function than in people with proteinuric CKD. Therefore, any changes in cardiac structure and function related to neprilysin inhibition, may have a greater impact on kidney function in patients with heart failure than with CKD.[200]

8.3.1 Glomerular haemodynamics in normal health

The kidney receives about a quarter of the circulating blood volume and filters about a fifth of this every minute.[296,297] Renal blood flow is provided by the renal artery, a branch of the abdominal aorta. This further subdivides into segmental arteries which become interlobar arteries and, in turn develop into arcuate arteries.[21,296] Afferent arterioles branch off from arcuate arteries and supply blood to the glomerulus of Bowman's capsule via the afferent arteriole which forms a capillary network.[21,296,297]

Efferent arterioles take blood away from the glomerulus and subdivide into peritubular capillaries, which then form the venous system which takes blood away from the kidney via the renal vein.[21,296] Renal blood flow is regulated by changes in the resistances of the renal artery and vein.[21,296] Renal blood flow (RBF) is calculated from renal plasma flow (RPF) and haematocrit using the formula:[297]

$$\text{RBF} = \frac{\text{RPF}}{(1\text{-haematocrit})}$$

GFR is the amount of fluid filtered from the glomerulus into Bowman's capsule (expressed as mL/min). GFR is determined from the capillary filtration coefficient (K_f), hydrostatic pressure in glomerular capillaries (P_{GC}) and Bowman's space (P_{BC}), the oncotic pressures of blood in the glomerular capillaries blood (π_{GC}) and Bowman's space (π_{BC}) and, glomerular plasma flow rate, using the formula:[21,296]

$$\text{GFR} = K_f ([P_{GC} - P_{BC}] - [\pi_{GC} - \pi_{BC}])$$

Glomerular capillary hydrostatic pressure is determined by afferent and efferent arteriolar resistance and the pressure within the renal artery.[21] GFR depends on net filtration pressure (which is usually between 10-16 mmHg), the surface area available for filtration and permeability of the glomerular basement membrane.[21] In healthy individuals, renal plasma flow (determined by the formula: [renal arterial pressure minus renal venous pressure] divided by total renal vascular resistance) is about 600-650 mL/min and GFR is between 100-140 mL/min.[296]

Filtration fraction (FF) is the fraction of renal plasma flow (RPF) filtered across the glomerulus and therefore the composition of renal tubular blood flow.[297,298] It is calculated using the formula:[297]

$$\text{FF} = \frac{\text{GFR}}{\text{RPF}}$$

GFR and renal blood flow are tightly regulated by “autoregulation”, independent of renal perfusion pressure, between 80-180 mmHg.[299] Autoregulation is mediated by tubuloglomerular feedback and the sympathetic nervous system.[299-301] The sympathetic nervous system innervates juxtaglomerular cells in the macula densa in the distal nephron to regulate renin release.[302] Sympathetic nerves also innervate proximal tubular cells and afferent and efferent arterioles with preferential regulation of afferent arteriolar vessel tone.[300,301]

If renal perfusion pressure increases, sodium reabsorption in the proximal tubule falls leading to greater sodium delivery to the macula densa.[302] The increased sodium load triggers afferent arteriolar vasoconstriction and reduced renin release.[300,301] Single nephron blood flow falls due to increased vascular resistance (due to reduced renal blood flow) and urinary sodium excretion.[299] The net effect of these changes is a reduction in single nephron GFR to maintain constant GFR and renal blood flow.[299]

When GFR is reduced, tubular fluid flow rate and sodium delivery falls and renin release increases causing arteriolar vasodilatation, arise in glomerular capillary hydrostatic pressure and an increase in GFR.[296,302] Renin acts on angiotensinogen to convert this into angiotensin I which is then converted to ATII by ACE. ATII acting on efferent arterioles produces vasoconstriction and in the adrenal cortex it stimulates the release of aldosterone from the adrenal glands.[297] Increased sympathetic activity augments proximal tubular sodium reabsorption and reduces urine volume.[301,302]

Changes in vessel diameter of the afferent or efferent arterioles alters glomerular hydrostatic pressure which drives ultrafiltration. Afferent arteriolar vasoconstriction decreases renal blood flow and GFR.[297] Efferent arteriolar vasoconstriction also reduces renal blood flow, but increases glomerular capillary hydrostatic pressure and filtration fraction.[297]

There are several neurohormonal determinants of glomerular haemodynamics. Efferent arteriolar vasoconstriction is augmented by ATII, endothelin-1, thromboxane A2 and reactive oxygen species.[21,296,303] Mediators of afferent arteriolar vasodilatation include NPs, nitric oxide (which increases renal plasma flow and GFR), prostaglandins (which vasodilate afferent and efferent arterioles increasing renal plasma flow) and bradykinin.[21,296,303] In addition to regulation of NPs, NEP is responsible for processing and breakdown of vasoactive peptides including prostaglandin, nitric oxide, bradykinin, ATII and endothelin-1. NPs result in natriuresis, diuresis, RAS inhibition (including suppression on renin and aldosterone

release) and reductions in systemic blood pressure.[304] NPs preferentially vasodilate the afferent arteriole to increase renal blood flow and augment glomerular capillary hydrostatic pressure and GFR. GFR may also be regulated through possible effects of NPs on glomerular permeability and on contractile elements in mesangial cells resulting in altered glomerular filtration surface area.[135]

8.3.2 Glomerular haemodynamics and renin-angiotensin system in progressive heart failure

In studies of patients with severe heart failure, individuals with mild renal impairment (GFR between 60 and 70 mL/min/m^2) associated with reduced cardiac output, exhibited compensatory increases in ultrafiltration (filtration fraction) which preserves GFR and intracapillary pressure, despite reductions in renal blood flow.[205,305,306] Patients with the most severely impaired cardiac function and, consequently GFR (around 40 mL/min/min^2), exhibited much smaller increases in filtration fraction.[305]

In heart failure renal blood flow is reduced to a greater amount proportionally than cardiac output.[306] In mild to moderate heart failure, filtration fraction of renal blood flow increases allowing a constant GFR to be maintained.[307,308] As heart failure progresses and systemic blood pressure falls, GFR becomes much more dependent on renal blood flow and perfusion pressure.[305] In order to maintain GFR, RAS activation produces marked efferent arteriolar vasoconstriction relative to the afferent arteriole mediated by ATII. This increases post-glomerular resistance and capillary hydrostatic pressure in spite of overall decreased renal perfusion pressure.[205,305,307]

In the most severe and advanced heart failure there is excessive renal afferent arteriolar vasoconstriction due to overwhelming activation of RAS, sympathetic nervous system and counterregulatory activation of neurohormonal responses including upregulation of the NP system and renal prostaglandin release (producing a vasodilatory response).[305,309] These mechanisms are aimed at increasing pre-glomerular resistances to maintain systemic blood pressure and prioritise delivery of circulating blood volume back to the heart and brain.[308]

Activation of the sympathetic nervous system stimulates sodium reabsorption and renin release which further amplifies the production and actions of ATII resulting in predominant afferent arteriolar vasoconstriction.[310] The combined effects of afferent and efferent arteriolar vasoconstriction reduces renal blood flow and intraglomerular pressure further.[290,292] The net effect of all the changes described is a fall in filtration

fraction and GFR.[307,309] ATII release also stimulates aldosterone and ADH production which further increases circulating blood volume through sodium and water retention in response to renal hypoperfusion and reduced circulating plasma volume in an attempt to sustain cardiac output and renal perfusion.[308]

8.3.2.1 *Effect of renin-angiotensin system inhibition on glomerular haemodynamics in heart failure*

RAS inhibition in heart failure makes GFR dependent on systemic blood pressure. RAS inhibition reduces systemic blood pressure and helps to reverse afferent arteriolar vasoconstriction and decrease post-glomerular resistance by blocking ATII-mediated efferent arteriolar vasoconstriction.[305,310] These changes result in predominant efferent arteriolar vasodilatation.[298] Capillary hydrostatic pressure is reduced which lowers filtration fraction and GFR falls (detectable as a rise in serum creatinine).[205,305,310]

In randomized trials of RAS inhibition in heart failure, post hoc analyses have not demonstrated adverse outcomes associated with this acute reduction in GFR.[311] Additionally, RAS inhibition did not reduce rates of long term decline in kidney function (over a 3 year follow-up period), compared with placebo.[312]

Results from randomized trials assessing the effects of RAS inhibition on kidney function in people with heart failure contrast to those with diabetic nephropathy or other proteinuric kidney diseases.[39-41] It is hypothesised that reduction in proteinuria with RAS inhibition is an important mechanism by which progressive decline in kidney function is prevented in people with CKD.[312] However, kidney disease associated with heart failure is not typically a proteinuric condition, therefore beneficial effects of RAS inhibitors may be less pronounced, particularly effects on decline in kidney function mediated through an effect on proteinuria.[312]

8.3.2.2 *Effect of neprilysin inhibition on glomerular haemodynamics in heart failure*

The addition of neprilysin inhibition to RAS inhibition, raises circulating NP concentrations which further reduces systemic blood pressure that is transmitted to the renal circulation as a significant reduction in renal perfusion pressure.[143] In animal models, ANP has been shown to produce vasodilatation of afferent arterioles, reducing pre-glomerular resistance, whilst simultaneously vasoconstricting efferent arterioles and increasing post-glomerular resistance.[117]

NEP inhibition has been shown to be associated with both upregulation and downregulation of endothelin-1.[199] The degree of NEP inhibition is likely to determine whether a predominant vasodilatory or vasoconstrictive effect on renal and systemic vasculature occurs. NPs also reduce sympathetic nervous system activity further enhancing the afferent arteriolar vasodilatory effects.[313]

8.3.2.3 *Effect of angiotensin receptor-neprilysin inhibition on glomerular haemodynamics in heart failure*

The combined actions of inhibition of RAS and NEP on efferent arterioles results in a relative vasoconstriction and an increase in post-glomerular resistance.[205,308] The overall effect of NPs on renal blood flow and GFR depends on the balance of afferent arteriolar vasodilatation and efferent arteriolar vasoconstriction.[313] Therefore, GFR will vary accordingly dependent on the actions of NPs on glomerular haemodynamics. In heart failure populations, combined NEP/RAS inhibition produces a rise in intracapillary hydrostatic pressure which increases filtration fraction in spite of considerably reduced systemic blood pressure and allows GFR to be maintained.[205,313]

8.3.3 Glomerular haemodynamics in CKD

Progressive CKD is associated with loss of functioning glomeruli and nephrons with resulting glomerular hyperfiltration.[14,23] In an attempt to minimise reductions in GFR that would otherwise occur due to reduced overall nephron mass, single nephron GFR increases due elevations in glomerular capillary hydrostatic pressure and decreased pre- and post-glomerular arteriolar resistances.[16,23,299] There is structural and functional hypertrophy of remaining nephrons which leads to the development of progressive proteinuria, tubulointerstitial fibrosis, glomerulosclerosis (which further increases glomerular permeability and proteinuria) and glomerular hypertension (with or without secondary systemic hypertension).[14,23,299] There is gradual loss of cortical glomeruli by global sclerosis and loss of glomerular capillaries with formation of direct connections between afferent and efferent arterioles (“aglomerular arterioles”).[314]

8.3.3.1 *Effects of renin angiotensin system inhibition on glomerular haemodynamics in CKD*

In animal models of CKD, micropuncture studies demonstrated treatment with ACE inhibitors substantially lowered systemic blood pressure and glomerular capillary hydrostatic pressure, resulting in significant reductions in proteinuria, glomerulosclerosis and hyperfiltration.[35,315,316] These findings were seen even after

ACE inhibition was delayed until the development of systemic hypertension.[35,315,316] In contrast, other antihypertensive agents that did not affect glomerular capillary pressure, were not associated with additional renoprotection.[315,316] Control of glomerular hypertension was deemed to be an essential mechanism for slowing the progression of renal disease.

In randomized clinical trials, long-term RAS inhibition significantly reduced the risk of progression to ESKD compared with placebo or other antihypertensive drug classes.[39-41,54] Following initiation of RAS inhibitors, there is an acute drop in GFR which is due to their haemodynamic effects, mediated by blocking the actions of ATII on the efferent arteriole which causes efferent arteriolar vasodilatation and so reducing glomerular capillary hydrostatic pressure.[317] Post-hoc analyses of trials of RAS inhibition, suggested that this acute reversible decline may be an indicator of subsequent rate of longer term decline in kidney function with a larger acute decline being associated with a slower long term rate of GFR decline (independent of blood pressure and albuminuria).[317] However, given the post-hoc nature of these analyses, comparisons of non-randomized changes in GFR were must interpreted with caution.

8.3.3.2 *Possible effect of neprilysin inhibition on glomerular haemodynamics in CKD*

In animal models of CKD, micropuncture studies showed combined NEP/RAS inhibition resulted in significantly greater reductions in glomerular capillary hydrostatic pressure compared with isolated RAS blockade.[138,159] The reductions may be in part due to preferential vasodilation of the afferent arteriole by NPs producing natriuresis, diuresis, vasodilatation and inhibition of RAS. These combined actions also significantly reduce systemic blood pressure and renal perfusion pressure. Greater lowering of glomerular capillary hydrostatic pressure with combined NEP/RAS inhibition translated into significantly decreased glomerulosclerosis, tubulointerstitial fibrosis and proteinuria compared with isolated RAS inhibition.[138,159]

8.3.4 Differences in effects on GFR with ARNIs between people with heart failure and those with CKD

Differences in the effects of ARNIs in people with CKD and heart failure are likely to be mediated through differences in the underlying mechanisms affecting renal haemodynamics. In heart failure, the overall effect of ARNIs is to increase capillary hydrostatic pressure to either maintain or increase GFR in the presence of reduced renal perfusion pressure. However, in CKD a reduction in capillary hydrostatic

pressure is the proposed mechanism by which RAS inhibitors confer their beneficial effects in slowing the rate of decline in GFR and progression of CKD.

NPs act directly on afferent arterioles and by blocking sympathetic activity to produce afferent arteriolar vasodilatation. They may also produce relaxation of mesangial cells to increase the surface area for filtration. As CKD progresses, the numbers of functioning nephrons decrease due to sclerosis, fibrosis and atrophy. However, this is quite different to the mechanism of renal impairment seen in people with CKD compared to those with heart failure.

It is plausible that once a certain degree of scarring, fibrosis and nephron loss has developed, kidneys of people with CKD are less responsive to the actions of NPs. Renal autoregulatory mechanisms are also likely to be impaired in CKD resulting in a decline in filtration fraction as opposed to a compensatory increase seen in people with heart failure.[309] Therefore, the size of the effect of NEP inhibition on kidney function could be much smaller in people with CKD compared to those with heart failure.

Animal models of cardiac disease, including CVD in CKD, have also suggested combined NEP/RAS inhibition may attenuate cardiac remodelling and improve cardiac function by limiting cardiac fibrosis and hypertrophy.[207,208,318] Over time, improvements in cardiac function may significantly enhance renal blood flow and GFR.[298]

The results from the UK HARP-III trial suggest that neprilysin inhibition with sacubitril/valsartan has similar efficacy to isolated RAS inhibition in maintaining renal function, at least in the short-term. However, a much larger trial and, of longer duration than UK HARP-III is required in people with advancing CKD, to address whether sacubitril/valsartan can preserve kidney function and the degree to which it can be maintained long-term, as suggested by the data among people with heart failure.

8.4 Effects of sacubitril/valsartan on albuminuria

Sacubitril/valsartan, compared with irbesartan, was associated with a non-significant reduction in albuminuria and a marginal trend towards lower levels of albuminuria at every follow-up visit. Although, the reduction in albuminuria did not reach statistical

significance, is it still of huge importance that albuminuria did not rise during the 12 month trial duration in a CKD population. This contrasts with uACR changes among patients with HFpEF where albuminuria increased by 50% in those allocated sacubitril/valsartan compared with valsartan and, by 20% in those with HFrEF allocated sacubitril/valsartan compared with enalapril.[200,202]

The UK HARP-III albuminuria results were similar across a broad range of pre-specified subgroups including: age; sex; blood pressure; presence of diabetes; prior vascular disease; BMI; kidney function; urinary sodium excretion and; prior use of RAS blockade. The effect of sacubitril/valsartan appeared to differ by underlying cause of CKD however, the source of heterogeneity in this subgroup was a 33% reduction in albuminuria seen in people with hypertensive or renovascular disease. This result is likely to be a chance finding arising as a consequence of multiple hypothesis testing and should be interpreted with caution given the overall result of no effect of sacubitril/valsartan on albuminuria.[236,256,268,319,320]

The albuminuria results from UK HARP-III are also not consistent with the findings from animal models of renal disease, in which combined NEP/RAS inhibition led to substantially lower levels of albuminuria compared with isolated RAS inhibition, despite similar reductions in blood pressure.[159] In these animal models, the subsequent rate of rise in albuminuria to pre-treatment levels was much slower compared with isolated RAS inhibition, suggesting a delay in progression to ESKD mediated by mechanisms beyond blood pressure reduction.[159] The reductions in albuminuria may have contributed to the improvements in renal structure and levels of pro-inflammatory cytokines and vasoactive peptides producing greater reductions in glomerulosclerosis, tubulointerstitial fibrosis and atrophy seen on histology from animals treated with combined NEP/RAS inhibition compared with isolated RAS inhibition.[138,158,159,161,295]

In a randomized trial of 1328 patients with mild-moderate hypertension, full dose treatment with sacubitril/valsartan reduced uACR levels by 12% (95% CI -25 to 4%) compared with baseline, but not more than the equivalent dose of valsartan (mean reduction in uACR of 10% [95% CI -24 to 8%]).[190] Although, albuminuria was extremely low in these participants at baseline and within the normal range, it is reassuring that the UK HARP-III results are consistent with these.

The effect of sacubitril/valsartan on albuminuria in UK HARP-III contrasts with results from heart failure populations. In people with HFpEF and HFrEF, sacubitril/valsartan

was associated with significant increases (although within the normal range) in albuminuria, compared with isolated RAS inhibition.[195,200,202,206] In PARAMOUNT, allocation to sacubitril/valsartan was associated with an increase in geometric mean uACR of 51.8% at 36 weeks compared with baseline but, remained stable in those allocated valsartan (difference in means of 0.9 mg/mmol at 36 weeks; P for difference = 0.016).[200] In PARADIGM-HF, albuminuria was higher in those allocated sacubitril/valsartan, compared with enalapril (difference in means of 0.3 mg/mmol).[202] If similar proportional increases in albuminuria with sacubitril/valsartan had been observed in people with proteinuric CKD, this would be of considerable concern since albuminuria has been shown to be associated with increased risk of progression to ESKD.[4,29,31,321]

Differences in the acute effects of sacubitril/valsartan on albuminuria between people with proteinuric CKD and those with heart failure may be mediated by the additional actions of NPs in the kidney. In animal models and patients with diabetes, ANP infusions were associated with increases in glomerular pressure, filtration fraction, urinary albumin excretion and changes in glomerular permeability.[134,135,322,323]

When NEP inhibition is combined with RAS inhibition, the additional reductions in systemic blood pressure and renal perfusion pressure result in preferential pre-glomerular arteriolar vasorelaxation and relative vasoconstriction of post-glomerular arterioles.[205] The resultant changes reduce glomerular resistance which increases capillary hydrostatic pressure in spite of an overall reduction in systemic blood pressure and renal perfusion pressure.[135,205] The changes in glomerular haemodynamics, permeability and renal arterial tone induced by NPs, further increases filtration fraction and GFR.[135,205] GFR may also be increased through possible effects of NPs on relaxation of mesangial cells resulting in an increased glomerular filtration surface area.[135] When such effects are combined with the direct effects of NPs on glomerular permeability, transcapillary albumin leak is increased, manifesting as increases in albuminuria.[135]

It is hypothesised that the aetiology of the increase in albuminuria, which normalises on withdrawal of sacubitril/valsartan in people with heart failure but not in those with proteinuric CKD, may be mediated by changes in renal haemodynamics and the balance of neurohormonal peptides.[135,202] People with CKD develop irreversible glomerular damage and resultant hyperfiltration which is not the mechanism underlying renal impairment in people with heart failure.[298]

8.5 Effects of sacubitril/valsartan on blood pressure

In UK HARP-III, sacubitril/valsartan significantly reduced systolic and diastolic blood pressure compared with irbesartan. Blood pressure was significantly lower with sacubitril/valsartan at every time-point except at 12 months, when the observed difference between the two treatments in blood pressure was no longer significant. The average number of additional anti-hypertensive medications taken by participants did not change throughout the trial.

In animal studies of combined NEP/RAS inhibition, mean arterial pressure was reduced by a similar degree to isolated RAS-inhibition.[138,159] Despite this, at each level of mean arterial pressure combined NEP/RAS inhibition reduced glomerular capillary pressure to a greater degree than isolated RAS inhibition resulting in increased renal plasma flow and reduced filtration fraction.[159,324] The actions of NPs and vasoactive peptides on renal haemodynamics may have afforded superior renoprotection.

Blood pressure reductions, similar to those in UK HARP-III, with sacubitril/valsartan compared with isolated RAS inhibition, have been demonstrated in trials of patients with hypertension and heart failure.[190-192,194,195] In patients with hypertension, full dose treatment with sacubitril/valsartan was associated with reductions in mean sitting systolic and diastolic blood pressure of 6.01 (95% CI -9.01 to -3.02) mmHg and 2.70 (95% CI -4.61 to 0.80) mmHg respectively, compared with the equivalent valsartan dose (320 mg).[190] The blood pressure reductions in UK HARP-III are consistent with the reductions achieved in this hypertension trial despite UK HARP-III participants only taking on average one other antihypertensive medication in addition to study treatment.

In the PARAMETER trial of 454 elderly patients with hypertension and arterial stiffness, compared with olmesartan, sacubitril/valsartan reduced central aortic systolic pressure by 3.7 mmHg (P=0.010), central aortic pulse pressure by 2.4 mmHg (P<0.012), mean 24-hour ambulatory brachial and central aortic systolic pressure by 4.1 and 3.6 mmHg (P<0.001).[192] In 114 patients with hypertension and raised pulse pressure, 52 weeks treatment with sacubitril/valsartan reduced left ventricular mass index more than olmesartan (mean difference 3.27 [95% CI 6.21-0.34] g/m^2; P=0.029) and central pulse pressure (mean difference 3.50 [95% CI -6.15 to -0.85] mmHg; P=0.010).[325] There was no significant difference in central aortic blood pressure.[325] These results suggest sacubitril/valsartan may have additional benefits on vascular

remodelling.[192] Progressive CKD is associated with vascular stiffening and calcification, so any effects ARNIs may have on vascular remodelling could be hugely beneficial to patients with CKD.[192]

Observational studies have shown raised blood pressure to be an independent risk factor for developing ESKD and the risk increases with rising blood pressure.[49,326] In people with advanced CKD, higher blood pressure is associated with faster rates of decline in GFR.[49,327,328] However, individual randomized trials of intensive blood pressure lowering in patients with advanced CKD have not shown this strategy to prevent progression of renal disease.[54,55]

A meta-analysis of intensive blood pressure lowering (including 9287 patients with CKD) suggested this intervention reduced the risk of the composite renal failure outcome (50% decline in GFR and doubling of the serum creatinine or ESKD) by 18% (HR 0.82; 95% CI 0.68-0.98) and, reduced the risk of ESKD-alone by 21% (HR 0.79; 95% CI 0.67-0.93).[57] Subgroup analysis found significant heterogeneity in the effect of intensive blood pressure lowering by baseline proteinuria, with a 27% (HR 0.73; 95% CI 0.62-0.86) reduction in the composite renal failure outcome in those with proteinuria and no apparent benefit in those without proteinuria (HR 1.12; 95% CI 0.67-1.87; P for heterogeneity = 0.006).[57]

A meta-analysis of 44,989 participants showed no reduction in risk of ESKD with intensive blood pressure lowering (RR 0.90; 95% CI 0.77-1.06) but, did reduce the risk of progression of albuminuria by 10% (95% CI 3-16).[329] A much larger meta-analysis including 613,815 participants, showed each 10 mmHg reduction in systolic blood pressure was associated with a non-significant 5% (RR 0.95; 95% CI 0.84-1.07) reduction in risk of ESKD.[58] At present it remains unclear whether the combined effects of a greater blood pressure lowering effect and reductions, albeit small, in albuminuria achieved with sacubitril/valsartan in UK HARP-III, could translate into reductions in risk of progression of CKD.

CKD is also a cause of hypertension which is associated with increased risk of cardiovascular disease.[8] Observational analyses have demonstrated that even among people with CKD at lowest probability of risk of CVD, each 10 mmHg rise in usual systolic blood pressure resulted in a 27% (HR 1.27; 95% CI 1.11-1.44) increase in cardiovascular risk.[92] The association between systolic blood pressure and risk of CVD events was similar for atherosclerotic (HR 1.25; 95% CI 1.06-1.48) and nonatherosclerotic events (HR 1.31; 95% CI 1.09-1.57).[92]

Meta-analyses in people with mild-to-moderate CKD have shown lowering blood pressure reduces cardiovascular risk in this population.[330] In a meta-analysis of 152,290 participants, including 30,295 with an eGFR less than 60 mL/min/1.73m^2, each 5 mmHg reduction in systolic blood pressure was associated with a 17% (HR 0.83; 95% CI 0.76-0.90) reduction in rates of major CV events (defined as stroke, coronary heart disease, heart failure and cardiovascular mortality). The effects were similar amongst people with CKD and an eGFR between 45 and 59 mL/min/1.73m^2 (HR 0.86; 95% CI 0.78-0.95) and those with an eGFR less than 45 mL/min/1.73m^2 (HR 0.86; 95% CI 0.73-1.02) with no heterogeneity in the effect compared with people with an eGFR of 60 mL/min/1.73m^2 or greater (P for heterogeneity = 0.93).[330]

A further meta-analysis of 613,815 participants, including 8769 participants with CKD, showed a similar 16% (RR 0.84; 95% CI 0.73-0.96) reduction in risk of major cardiovascular events in people with CKD but, a larger proportional reduction in people without CKD (RR 0.68; 95% CI 0.62-0.75; P for heterogeneity = 0.012).[58] Each 10 mmHg reduction in systolic blood pressure was associated with a non-significant 5% (RR 0.95; 95% CI 0.84-1.07) reduction in the effect on the renal composite outcome (defined as ESKD leading to initiation of dialysis, transplantation, or death).[58]

In animal models of cardiovascular disease, treatment with sacubitril/valsartan reduced aortic fibrosis and markers of cardiac hypertrophy and fibrosis (fibroblast growth factor [FGF]-23 and NT-proBNP), and improved markers of cardiac oxidative stress and inflammation, to a greater degree than isolated RAS inhibition.[207,208,318] The blood pressure lowering effects observed in these animal studies and from heart failure trials suggest sacubitril/valsartan could help reduce the risk of cardiovascular events observed in patients with CKD.

8.6 Effects of sacubitril/valsartan on cardiac biomarkers

As CKD progresses, the manifestation of CVD changes from predominately atherosclerotic disease, for example myocardial infarction and ischaemic stroke, to non-atherosclerotic disease, characterised by arteriosclerosis (due to the development of vascular calcification) and structural heart disease (for example LVH and increased LV mass) which manifests clinically similar to heart failure with a high incidence of sudden cardiac death.[9,72,86-88,331]

The utility of cardiac biomarkers including troponin and NT-proBNP in patients with CKD and ESKD has been questioned as their concentrations may be affected by the degree of renal impairment due to impaired renal clearance (particularly NT-proBNP), as well as underlying cardiac disease and volume status.[332,333] However, studies have shown that even after adjustment for eGFR and albuminuria, these biomarkers remain independent predictors of CVD (symptomatic and asymptomatic) in patients with CKD.[334,335] Furthermore, NT-proBNP is not metabolised by neprilysin (unlike BNP) so levels are not affected by NEP inhibition and can be used as a measure of cardiac disease and dysfunction in patients treated with sacubitril/valsartan.[336]

In people with CKD, troponin has been shown to predict the development of heart failure[337] and correlate with left ventricular (LV) mass[338-340] and cardiac function.[341] Troponin I concentrations have been used to risk stratify individuals with CKD without a known history of CVD. In 7278 patients with CKD without CVD, increasing baseline troponin I was strongly associated with future CVD risk.[92] Compared with people with an undetectable troponin I concentration (0.01 ng/mL or less) individuals with substantially elevated troponin I (greater than 0.03 ng/mL) had nearly a 3-fold increase in CVD risk (HR 2.82; 95% CI 2.42-3.28).[92] Raised troponin-I was associated with increased CVD risk in both non-dialysis and dialysis patients.[92]

NT-proBNP has been used a marker of LV mass,[334,339] LV hypertrophy,[334,339,340] LV systolic function[339] and heart failure in CKD.[337] In the general population, NT-proBNP is used for the diagnosis, management[342] and prognosis of heart failure[343,344] and increasing concentrations have been associated with adverse cardiovascular and mortality outcomes.[344-346] NT-proBNP and troponin have both been used to predict risk of mortality and cardiovascular events in the CKD and ESKD population as well as in the general population.[333,346,347]

Elevated concentrations of both these biomarkers may also be predictive of an increased risk of progression of CKD (HR per SD increase in log high sensitivity troponin 1.11 [95% CI 1.01-1.22] and, HR per SD increase in log NT-proBNP 1.24 [95% CI 1.13-1.36])[337,348] and, risk of adverse cardiovascular outcomes in people with CKD even after adjustment for eGFR.[349-351]

In UK HARP-III, sacubitril/valsartan significantly reduced concentrations of NT-proBNP by 18% (95% CI -25 to -11%; P<0.001) and troponin I by 16% (95% CI -23% to -8%; P<0.001), compared with irbesartan. Similar reductions in these biomarkers have also been seen shown in patients with HFpEF,[195,211] HFrEF and hypertension

treated with sacubitril/valsartan.[192] The reductions in circulating concentrations of NT-proBNP and troponin with sacubitril/valsartan in patients with CKD in UK HARP-III are most likely related to the actions of increasing concentrations of circulating NPs with NEP inhibition resulting in: natriuresis; diuresis; inhibition of RAS and sympathetic nervous system activation and; possible anti-fibrotic and anti-hypertrophic effects on the heart.[99]

The cardiac biomarker results from UK HARP-III raise the hypothesis that, as in heart failure trial populations,[194,195] sacubitril/valsartan may have beneficial effects on cardiac structure and function and subsequently cardiovascular outcomes in patients with CKD. Since CVD mortality increases as CKD progresses,[9] if sacubitril/valsartan had similar cardiovascular effects in people with CKD to those seen in PARADIGM-HF, sacubitril/valsartan could substantially improve CVD outcomes in CKD.[194]

In trials of heart failure, the greater blood pressure lowering effects seen with sacubitril/valsartan did not correlate with the treatment effect on cardiovascular outcomes.[194,196] These results suggest that the beneficial cardiovascular effects seen with sacubitril/valsartan in patients with CKD may be mediated through additional mechanisms beyond just blood pressure lowering. However, the blood pressure and biomarker results with sacubitril/valsartan (compared with isolated RAS inhibition) in UK HARP-III are hypothesis generating and provide a rationale to support a much larger cardiovascular outcomes trial in people with CKD.

8.7 Pharmacokinetics of sacubitril/valsartan in CKD

Pharmacokinetic analyses were undertaken to assess the determinants of plasma concentrations of metabolites of sacubitril/valsartan in individuals with advanced CKD. The only significant determinant of sacubitrilat concentration was kidney function. Each 10 mL/min reduction in mGFR, resulted in 1485 ng/mL higher serum concentrations of sacubitrilat (the active form of sacubitril; P=0.002). Sacubitrilat is predominantly renally excreted, so it is not surprising that impaired or declining renal function results in higher sacubitrilat concentrations compared with people with normal kidney function.[184,185]

In a pharmacokinetic study of sacubitril/valsartan in 24 people with CKD, higher steady-state serum concentrations of sacubitrilat correlated with the degree of renal

impairment.[185] In UK HARP-III, higher concentrations of sacubitrilat may have contributed to the lower blood pressure seen in those allocated to sacubitril/valsartan. Reassuringly, there was no excess of side effects such as derangements in liver function or angioedema in a population with advanced CKD and higher sacubitrilat concentrations.

8.8 Safety of sacubitril/valsartan

Sacubitril/valsartan, compared with irbesartan, did not generate any major safety concerns in people with progressive CKD. Rates of fatal and non-fatal serious adverse events were similar and importantly there were no excess serious adverse events of angioedema, hypotension or requirement for dialysis with sacubitril/valsartan compared with irbesartan.

8.8.1 Angioedema

Only one adverse event of angioedema occurred during the trial, in a participant allocated sacubitril/valsartan. This resolved spontaneously without any treatment and the participant did not seek any medical attention. When the adverse event was reported, I contacted the participant to collect additional detailed information relating to the event to compile an adverse event report. I advised the participant to stop all study treatment immediately and to restart their pre-trial ARB. The participant continued to attend follow-up visits for the remainder of the trial. The event report was sent to the DMC, the trial sponsor, the University of Oxford, and the manufacturer of sacubitril/valsartan (Novartis). Only the DMC were provided with the unblinded treatment allocation.

Similar safety data have been demonstrated in other trials of sacubitril/valsartan.[190,194] In PARADIGM-HF, there was no difference in the rates of angioedema between sacubitril/valsartan and enalapril (19/4187 versus 10/4212 events respectively).[194] However, in PARAGON-HF, angioedema rates were significantly higher in those allocated sacubitril/valsartan compared with valsartan (14/2407 [0.6%] versus 4/2389 [0.2%]; P=0.02) but no participants developed airway compromise.[196] In UK HARP-III there were not enough events of angioedema to fully assess what the effect of sacubitril/valsartan on angioedema in people with CKD might be. A much larger trial

and of longer duration of exposure to sacubitril/valsartan would be required to allow a more comprehensive assessment of the adverse event profile in CKD.

8.8.2 Hypotension

There were only two SAEs of hypotension with one occurring in each treatment group. Rates of non-serious hypotension were significantly higher with sacubitril/valsartan than with irbesartan (8.2% versus 3.4% respectively). This resulted in a small non-significant excess in numbers of participants allocated sacubitril/valsartan having their study treatment dose halved, compared with irbesartan (3% versus 1%). Importantly, there was no difference in the proportions of participants stopping sacubitril/valsartan completely. The reduction in study treatment dose was satisfactory to control symptoms of hypotension and maintain compliance with study treatment.

Trials in heart failure populations, comparing sacubitril/valsartan with isolated RAS inhibition have similarly shown higher rates of symptomatic hypotension (588/4187 [14%] versus 388/4212 [9.2%] respectively; P<0.001) including symptomatic hypotension with a systolic blood pressure of less than 90 mmHg (112/4187 [2.7%] versus 59/4212 [1.4%] respectively; P<0.001).[194] However, as in UK HARP-III, hypotension rarely resulted in discontinuation of sacubitril/valsartan.[194]

8.8.3 Renal safety

8.8.3.1 *Hyperkalaemia*

In UK HARP-III, sacubitril/valsartan had no significant effect on overall rates of hyperkalaemia (any potassium result of 5.5 or higher), compared with irbesartan. There was a slight numerical excess in the rates of moderate hyperkalaemia (potassium between 6.0 to 6.4 mmol/L) in participants allocated sacubitril/valsartan, compared with irbesartan. However, rates of severe hyperkalaemia (potassium 6.5 mmol/L or above) with sacubitril/valsartan were similar to irbesartan.

Only one serious adverse event related to hyperkalaemia was reported in UK HARP-III, in a participant assigned irbesartan. Non-serious adverse events of hyperkalaemia believed by the participant to be related to study treatment were reported in 2% of participants assigned sacubitril/valsartan and resulted in withdrawal of study treatment in all cases.

The potassium results from UK HARP-III contrast with those in heart failure trials of sacubitril/valsartan. Rates of hyperkalaemia with a potassium greater than 6.0 mmol/L were significantly lower with sacubitril/valsartan compared with enalapril (181/4187 [4.3%] versus 236/4212 [5.6%] respectively; P=0.007)[194] or valsartan (75/2386 [3.1%] versus 101/2367 [4.3%] respectively; P=0.04).[196]

The lower rates of hyperkalaemia in heart failure trials may relate to greater diuretic use (likely loop diuretics) and higher eGFR compared with UK HARP-III participants. Loop diuretics act in the thick ascending limb in the kidney (where about 25% of the filtered sodium is normally reabsorbed) and macula densa to inhibit the sodium-potassium-chloride-2 cotransporter which exchanges sodium and chloride ions for potassium.[352,353] This impedes sodium reabsorption and increases distal tubular sodium concentration causing a natriuresis and resultant osmotic diuresis.[352,353] In PARADIGM-HF 80% of participants in each treatment group were taking diuretics.[194] In UK HARP-III only about 40% of participants were taking diuretics and with worsening renal function there is a greater susceptibility to hyperkalaemia due to impaired potassium excretion and development of metabolic acidosis.[353]

Treatment with sacubitril/valsartan provides additional aldosterone and RAS inhibition compared with isolated RAS inhibition. Therefore, in patients with CKD in whom GFR is reduced, it is not surprising that UK HARP-III participants had higher serum potassium concentrations, perhaps as a result of greater RAS inhibition with sacubitril/valsartan compared with equivalent doses of irbesartan.

8.8.3.2 *Acute kidney injury*

In UK HARP-III, there was no difference in rates of acute kidney injury (defined as a 25% or greater reduction in CKD-EPI eGFR) between those allocated sacubitril/valsartan and those allocated irbesartan. The UK HARP-III results are in keeping with the renal safety data from other trials of sacubitril/valsartan among people with heart failure and hypertension, in which no excess of renal adverse events occurred.[194,196,202]

In the PARADIGM-HF trial, elevations in serum creatinine (creatinine 2.5 mg/dL [220 micromoles/L] or greater) occurred less frequently in participants allocated sacubitril/valsartan compared with enalapril (139/4187 [3.3%] versus 188/4212 [4.5%] respectively; P=0.007).[194] However, there was no difference in rates of more substantial reductions in serum creatinine (greater than 3.0 mg/dL [266.4 mg/mmol])

in participants allocated sacubitril/valsartan, compared with enalapril (63/4187 [1.5%] versus 83/4212 [2.0%] respectively; P=0.10).[194]

Overall, rates of discontinuation of study treatment due to renal adverse events were lower with sacubitril/valsartan than enalapril (29/4187 [0.7%] versus 59/4212 [1.4%] respectively; HR 0.49 [95% CI 0.3-10.76]; P=0.002).[202] Amongst people with CKD, significantly fewer participants allocated sacubitril/valsartan discontinued treatment for a renal cause, compared with enalapril (15/4187 [1.1%] versus 36/4212 [2.6%]; HR 0.43 [95% CI 0.24-0.80] respectively; P=0.008; P for interaction = 0.52).[202]

It is not surprising that occurrence of acute kidney injury was low in UK HARP-III or in other trials of sacubitril/valsartan. In UK HARP-III, 85% of participants were taking RAS-inhibition at screening and similar numbers in heart failure trials. The study populations selected included participants known to tolerate RAS blockade, reducing the risk of acute kidney injury (which can arise with RAS inhibition). The participants selected for the trials were generally well and stable with regards their heart failure or CKD. Finally, the effects on sacubitril/valsartan on renal haemodynamics particularly preferential relaxation of the afferent arteriole maintaining GFR despite reductions in systemic blood pressure and renal perfusion pressure induced by NPs, coupled with the effects of NPs on other vasoactive and neurohormonal substances are likely to have reduced the risk of acute kidney injury and renal adverse events.

8.8.4 Liver impairment

In UK HARP-III, no hepatic adverse events including transaminitis or more significant liver injury (e.g. fulminant hepatic failure) were observed with either sacubitril/valsartan or irbesartan. The safety data from UK HARP-III are extremely encouraging since the clearance of valsartan is predominantly via enterohepatic circulation. This route also accounts for about 35-50% of the clearance of sacubitrilat, concentrations of which significantly increased with decreasing kidney function.[184]

Randomized trials in hypertension and heart failure populations have not reported any excess cases of liver enzyme impairment or injury in participants allocated to sacubitril/valsartan compared with isolated RAS inhibition.[190,192,194,195] In PARAGON-HF, liver-related adverse event reports were similar between sacubitril/valsartan and valsartan (151/2407 [6.3%] versus 178/2389 [7.5%] respectively; P=0.11).[196]

The safety data from UK HARP-III provide important information regarding the use of sacubitril/valsartan in people with CKD. The results suggest sacubitril/valsartan could

potentially be safely prescribed in patients with a lower eGFR (down to 20 ml/min/1.73m^2) than currently licenced (eGFR greater than 30 ml/min/1.73m^2).[186] This would enable a greater proportion of patients with CKD and/or heart failure (and who are at greatest risk of adverse CV events) to benefit from treatment with sacubitril/valsartan.

8.9 Limitations

The UK HARP-III trial was a phase II, randomized trial of 414 participants with CKD with a short duration of follow-up (12 months) and exposure to sacubitril/valsartan. The trial had adequate power to examine the short-term effects of sacubitril/valsartan on kidney function however, the duration of follow-up and sample size was insufficient and, moreover the trial was not designed to assess clinical outcomes, such as risk of progression to ESKD or CV events in people with CKD.

The choice of the comparator, irbesartan, could have influenced the interpretation of the renal function results. Irbesartan was chosen as the comparator as it is licenced for the treatment of proteinuric kidney disease following the results of randomized trials showing that it reduced rates of progression of nephropathy compared with placebo or other antihypertensive medications.[41,354] However, valsartan is the ARB that is combined with sacubitrilat to form the trial drug sacubitril/valsartan. Both Irbesartan and valsartan have some differences in their bioavailability, half-life, affinity for the angiotensin-type 1 receptor and metabolism,[355,356] which may affect their anti-hypertensive and renal haemodynamic properties and contributed to the observed lack of effect on renal function.[355-357] It is more likely that the overall effects of both drugs are comparable and these differences are unlikely to translate into any material effect on kidney function.

The results of the effects of sacubitril/valsartan on blood pressure and cardiac biomarkers, although of significant interest, should only be regarded as hypothesis generating as these were exploratory outcomes and the trial was not powered to detect differences in such outcomes. A large-scale clinical outcomes trial would be required to adequately examine the CV and renal effects of sacubitril/valsartan in people with CKD.

8.10 Future prospects for angiotensin receptor-neprilysin inhibition in CKD

The UK HARP-III trial demonstrated ARNI with sacubitril/valsartan may offer a new therapeutic strategy to address the excess CVD risk in patients with CKD. The substantial reductions in blood pressure and cardiac biomarkers (troponin I and NT-proBNP), compared with isolated RAS-inhibition, were similar to those seen in heart failure trials.[194,195] If the reductions in blood pressure and cardiac biomarkers were of a similar magnitude to the reductions in CVD outcomes in people with CKD as in the general population, ARNIs could significantly improve the outlook for people with CKD. This is of particular importance since the manifestations of CVD associated with progressive CKD are similar to heart failure with vascular stiffness and arteriosclerosis rather than atherosclerotic disease.[72,86-88]

Sacubitril/valsartan did not demonstrate an effect on kidney function in people with CKD over 12 months, compared with isolated RAS inhibition. Importantly, there was no increase in albuminuria seen in patients with CKD, 65% of whom had macroalbuminuria, unlike in patients with heart failure.[194,195] The effects on kidney function and albuminuria are likely mediated via the actions of NPs on haemodynamic changes within the kidney.[135,205,305-309] In animal models these changes have translated into substantial reductions in histological features associated with progression of CKD, however the long-term effects of this in humans in uncertain.[138,158,159]

In people with diabetic nephropathy, randomized trials of SGLT-2 inhibition have demonstrated significant reductions in both cardiovascular (including cardiovascular mortality and fatal and non-fatal cardiovascular events) and renal outcomes (including progression to ESKD and renal mortality).[64,65,67] Effects of SGLT-2 inhibition in people with advanced non-diabetic CKD are currently unknown although trials are currently ongoing in this population to assess effects on CVD and renal outcomes.[68] Even if these trials show significant reductions in renal and cardiovascular outcomes, there is still an argument for a potential role of ARNI in CKD for cardiovascular benefit to treat any residual risk given ARNIs act at a different site to SGLT-2 inhibitors in the kidney and via a different mechanism of action.

The UK HARP-III trial results suggest that ARNI may have a much bigger effect on reductions in CVD outcomes in people with CKD than renal outcomes. The data support the need for a large-scale randomized clinical outcomes trial of ARNIs in people with CKD to assess primarily effects on CVD outcomes and any assessment of renal effects would likely be a secondary outcome.

8.11 Summary

The UK HARP-III trial is the first randomized trial to examine the effects of sacubitril/valsartan on kidney function in people with advanced CKD. UK HARP-III has shown that, 12 months of treatment with sacubitril/valsartan had similar effects to irbesartan on kidney function. Importantly, unlike in the heart failure population, sacubitril/valsartan had no adverse effect on albuminuria.

Sacubitril/valsartan significantly lowered blood pressure and concentrations of cardiac biomarkers troponin I and NT-proBNP, compared with irbesartan, suggesting that the drug could provide substantial benefit on cardiovascular outcomes (a major cause of morbidity and mortality in people with CKD) in people with advanced and progressive CKD, as in heart failure trials with this drug. Importantly, sacubitril/valsartan had similar tolerability to irbesartan, and no major safety concerns were observed.

There is currently an unmet clinical need for interventions that could both reduce the risk of progression of CKD to ESKD and CVD in people with CKD, especially non-diabetic renal disease. The results of UK HARP-III provide a strong rationale for undertaking a large-scale outcomes trials examining the effects of ARNI on cardiovascular outcomes in people with CKD. The trial did not exclude a potential benefit on kidney function and so effects of sacubitril/valsartan on progression of CKD to ESKD would also need to be considered in any future large-scale clinical outcomes trial in this population.

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Appendix 1:

Trials of renin-angiotensin system blockade and renal outcomes.

Study Name Acronym (year)	Study population	Active Treatment	Control	Mean age (years)	Number of ESRD events (active vs control)	ESRD analysis (95% CI)	Number of events for clinical composite analysis	Clinical composite Analysis (95% Confidence Interval)
Renin-angiotensin system blockade								
AASK (2002)[1]	1,094 African Americans aged 18-70, GFR 20-65 mL/min/1.73^2 with hypertensive nephrosclerosis	Ramipril (n=436)	Metoprolol (n=441)	54.4	47 vs 32	RR 22% (-10 to 45) P=0.16	GFR event, ESRD, or death = 126	RR 22% (1 to 38) P=0.04
AASK (2002)[1]	1,094 African Americans with hypertensive nephrosclerosis	Ramipril l217	Amlodipine 441	54.5	32 vs 47	RR 59% (36 to 74) P<0.001	GFR event, ESRD, or death = 56	RR 38% (14 to 56) P=0.004
AASK (2002)[1]	1,094 African Americans with hypertensive nephrosclerosis	Metoprolol (n=441)	Amlodipine 212	54.9	NA	RR 59% (36 to 74) P<0.001	GFR event, ESRD, or death = 155	RR 20% (-10 to 41) P=0.17
ADVANCE (2007)[2]	11,140 aged >55 years with type 2 diabetes, & history of major CVD or ≥1 other risk factor for CVD	Perindopril + Indapamide (n=5,569)	Placebo (n=5,571)	66	NA		New or worsening nephropathy + new macroalbuminuria	RRR 21% (15-27%) P<0.0001
AIPRI (1999)[3]	583 with CKD (Cr 133-354 µmol/L + 24hr estimated CrCl 30-60 ml/min)	Benazepril (n=300)	Placebo (n=283)	51	1 vs 1	NA	Doubling of SCr or need for dialysis = 31 vs 57	NA
ALTITUDE (2012)[4]	8,531 aged ≥35 years with type 2 diabetes, microalbuminura, macroalbuminuria or CVD	Aliskiren (n=4,274)	Placebo (n=4,287)	64.5	121 vs 113	HR 1.08 (0.84-1.40) P=0.56	ESRD or doubling of SCr from baseline = 257 vs 251	HR 1.03 (0.87-1.23) P=0.74
Benazepril (2006)[5]	422 Chinese aged 18-70 with CKD, SCr 133-442 µmol/L & CrCl 20-70 ml/min/1.73m^2; non-diabetic renal disease; & proteinuria (>0.3 g/d)	Benazepril Group 1 n=104	Placebo Group 2 only n=112	44.8	NA	NA	Doubling of SCr, ESRD or death = 44 vs 65	NA
CSG-Captopril (1993)[6]	409 aged 18-49 with IDDM and diabetic retinopathy, urine protein >500 mg/d & SCr <221 µmol/L	Captopril (n=207)	Placebo (n=202)	34.5	20 vs 31	NA	Dialysis, transplant or death = 23 vs 42	RRR 46% (10-68)
IDNT (2001)[7]	1715 >35 years with type 2 diabetes, hypertension (sitting SBP >135 mmHg, DBP > 85 mmHg), Urine protein ≥900 mg/d). SCr 88-265 µmol/L in women & 106-265 µmol/L in men	Irbesartan (I; n=579)	Amlodipine (A; n=567) and placebo (P; n=569)	58.9	I = 82 A = 104 P = 101	**I vs P** HR 0.83 (0.62-1.11) P=0.19 **I vs A =** HR 0.76 (0.57-1.02) P=0.06 **A vs P** HR 1.09 (0.82-1.43) P=0.56	Doubling in SCr, ESRD, or death = 644 in total: I = 189 A= 233 P = 222	**I vs P** HR 0.81 (0.67-0.99) P=0.03 **I vs A =** HR 0.76 (0.63-0.92) P=0.005 **A vs P** HR 1.07 (0.89-1.29) P=0.47

Effects of blood pressure lowering interventions on risk of end-stage renal disease and other renal outcomes

Study Name Acronym (year)	Study population	Active Treatment	Control	Mean age (years)	No of ESRD events (active vs control)	ESRD analysis (95% CI)	No of events for clinical composite analysis	Clinical composite Analysis (95% CI)
ONTARGET (2008)[8]	25,620, >55 years with atherosclerotic vascular disease or diabetes with end-organ damage. SCr <265 µmol/L	Telmisartan n=8542	Ramipril n=8576	66.4	51 vs 48	HR 1.07 (0.72-1.58) P=0.747	Dialysis, doubling in SCr, death 1147 vs 1150	HR 1.00 (0.92-1.09) P=0.968
ONTARGET (2008)[8]	25,620, >55 years with atherosclerotic vascular disease or diabetes with end-organ damage. SCr <265 µmol/L	Ramipril and Telmisartan (n=17,118)	Ramipril (n=8576)	66.4	63 vs 48	HR 1.33 (0.92-1.94) P=0.133	Dialysis, doubling in SCr, death 1233 vs 1150	HR 1.09 (1.01-1.18) P=0.037
ORIENT (2011)[9]	566 Japanese & Chinese aged 30-70, with T2 diabetes; UACR >33.9 mg/; SCr 88.40-221.00 µmol/l in women and 106.08-221.00 µmol/l in men.	Olmesartan (n=282)	Placebo (n=284)	59.2	74 vs 78	HR 1.08 (0.78-1.49)	Dialysis, doubling in SCr, death Renal composite outcome 116 vs 129	HR 0.97 (0.75-1.24)
REIN (1997)[10] = Stratum 2 results	352 Aged 18-70 with 166 in Stratum 2, CrCl 20-70 mL/min/1.73m^2 and persistent proteinuria, not received ACEi for at least 2 months.	Ramipril + conventional anti-hypertensives (n=38)	Placebo + conventional anti-hypertensives (n=49)	49.3	17 v 29	NA	Doubling of SCr or ESRD 18 vs 40	NA
REIN (1999)[11] = Stratum 1 results	352 Aged 18-70, with 186 in Stratum 1, CrCl 20-70 mL/min/1.73m^2 and persistent proteinuria, not received ACEi for at least 2 months.	Ramipril + conventional anti-hypertensives (n=99)	Placebo + conventional anti-hypertensives (n=87)	49.7	9 vs 18	RR 2.72 (1.22-6.08)	Decline in GFR per month (mL/min)	0.26 [SE 0.05] vs 0.25 [SE 0.06] mL/min P=0·52
RENAAL (2001)[12]	1,513 Aged 31-70, type 2 diabetes and UACR >300 mg/g and SCr 115-265 µmol/L.	Losartan	Placebo	60	147 vs 194	RRR 28% (11-42%) P=0.002	Doubling of SCr, ESRD, or death 327 vs 359	RRR 16% (2-28%) P=0.02
ROAD (2007)[13]	360 Chinese aged 18-70, with non-diabetic proteinuric CKD. SCr 133-442 µmol/L & CrCl 20-70 ml/min/1.73m^2	Up-titrated Benazepril (10-40mg/d)	Conventional dose (10mg/d) Benazepril	50.5	NA	RR 47% (4.2-72.1) P=0.042	Doubling of SCr, ESRD, or death 15 vs 26	RR 51% (4.8-73.3) P=0.028

Effects of blood pressure lowering interventions on risk of end-stage renal disease and other renal outcomes

Study Name Acronym (year)	Study population	Active Treatment	Control	Mean age (years)	No of ESRD events (active vs control)	ESRD analysis (95% CI)	No of events for clinical composite analysis	Clinical composite Analysis (95% CI)
ROAD (2007)[13]	360 Chinese aged 18-70, with non-diabetic proteinuric CKD. SCr 133-442 µmol/L and CrCl 20-70 ml/min/1.73m^2	Up-titrated Losartan (50-200mg/d)	Conventional dose (50mg/d) Losartan	51.5	NA	RR 47% (3.6-76.9) P=0.046	Doubling of SCr, ESRD, or death 13 vs 26	RR 53% (5.5 to 74.1) P=0.022
TRANSCEND (2008)[14]	5,926 with intolerance to ACEi and CAD, PVD or stroke, or diabetes with end-organ damage without evidence of heart failure	Telmisartan	Placebo	66.9	4 vs 6		Doubling of SCr or chronic dialysis 89 patients	Relative increase of 38% (-10% to +110%)
VA-NEPHRON D (2013)[15]	1,448 Veterans with type 2 diabetes, UACR ≥300 mg/g and eGFR 30-89.9 mL/min/1.73m^2	Losartan and Lisinopril (n=724)	Losartan and placebo (n=724)	64.6	27 vs 43	HR 0.66 (0.41-1.07) P=0.07	eGFR decline, ESRD, or death 132 vs 152	HR 0.88 (0.70-1.12) P=0.30

AASK = African American Study of Kidney Disease and Hypertension; ADVANCE = The Action in Diabetes and Vascular disease: Preterax and Diamicron-MR Controlled Evaluation; AIPRI = Angiotensin converting enzyme inhibition in renal insufficiency; ALTITUDE = The Aliskiren Trial in Type 2 Diabetes Using Cardiorenal Endpoints; CSG-Captopril = Collaborative Study Group - Captopril trial; IDNT = Irbesartan in Diabetic Nephropathy; ONTARGET = Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial; ORIENT = Olmesartan Reducing Incidence of Endstage Renal Disease in Diabetic Nephropathy Trial; REIN = Ramipril Efficacy in Nephropathy; RENAAL = Reduction of Endpoints in Non-Insulin Dependent Diabetes Mellitus (NIDDM) with the Angiotensin II Antagonist Losartan; ROAD = Renoprotection of Optimal Antiproteinuric Doses; TRANSCEND = Telmisartan Randomized Assessment Study in ACE Intolerant Subjects with Cardiovascular Disease; VA-NEPHRON = Veterans Affairs Nephropathy in Diabetes.

ACEi = angiotensin converting enzyme inhibitor; CAD = coronary artery disease; CKD = chronic kidney disease; CrCl = creatinine clearance; CVD = cardiovascular disease; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; GFR = glomerular filtration rate; ESRD = end-stage renal disease; HR = hazard ratio; IDDM = insulin-dependent diabetes mellitus; MAP = mean arterial pressure; PVD = peripheral vascular disease; RR(R) = relative risk reduction; SBP = systolic blood pressure; SCr = serum creatinine; UACR = urine albumin:creatinine ratio.

Effects of blood pressure lowering interventions on risk of end-stage renal disease and other renal outcomes

Intensive versus standard/usual blood pressure lowering								
Study Name Acronym (year)	**Study population**	**Active Treatment**	**Control**	**Mean age (years)**	**No of ESRD events (active vs control)**	**ESRD analysis (95% CI)**	**Clinical composite and No of events for analysis**	**Clinical composite Analysis (95% CI)**
AASK (2002)[1]	1,094 African Americans aged 18-70, GFR 20-65 mL/min/1.73^2 with hypertensive nephrosclerosis	INTENSIVE BP control (MAP <92 mmHg) with Ramipril (n=540)	STANDARD (MAP 102-107 mmHg) BP control with metoprolol or amlodipine (n=554)	54.6	83 vs 88	RR 6% (-29 to 31) P=0.72	GFR event, ESRD, or death = 340	RR 2% (-22 to 21) P=0.85
ESCAPE (2009)[16]	385 Children (3-18 years), GFR 15-80 & 24-hr MAP >95th percentile, or controlled by antihypertensive medication	Ramipril for INTENSIVE BP lowering (n=189)	Ramipril for a CONVENTIONAL BP target (n=196)	11.5	22 vs 34	NA	50% decline in GFR or ESRD = 46 vs 69	HR 0.65 (0.44-0.94) P=0.02
MDRD 1 (1994)[17, 18]	585 Aged 18-70, GFR 25-55mL/min/1.73^2, a MAP (calculated as 2/3 of DBP + 1/3 of SBP) of ≤125 mmHg and a dietary protein intake >0.9g/kg body weight/d.	LOW BP (MAP ≤92 mmHg if ≤60 years or ≤98 mmHg if >60 years) AND Usual protein (1.3g/kg/day) (n=145) diet OR Low protein diet (0.58g/kg/day)(n=140)	USUAL BP (MAP ≤107 mmHg if ≤60 years or ≤113 mmHg if >60 years) AND Usual protein diet (1.3g/kg/day) (n=145) OR Low protein diet (n=140)	52	MDRD 1+2 Low vs Usual = 61 vs 66	HR 0.76 (0.52-1.10) P=0.15	Rate of decline in GFR (slope)	GFR slope = 10.7 (9.1-12.4) mL/min vs 12.3 (10.6-14.0) mL/min
MDRD 2 (1994)[17, 18]	255 Aged 18-70, GFR 13-24 mL/min/1.73^2 & MAP ≤125 mmHg, irrespective of protein intake.	LOW BP (MAP ≤92 mmHg if ≤60 years or ≤98 mmHg if >60 years) AND Low protein diet (n=67) OR Very Low protein diet (0.28g/kg/day) (n=65)	USUAL BP (MAP ≤107 mmHg if ≤60 years or ≤113 mmHg if >60 years) (n=123) AND Low protein diet (n=62) OR Very Low protein diet (n=61)	52	MDRD 1+2 Low vs Usual = 61 vs 66	HR 0.76 (0.52-1.10) P=0.15	Rate of decline in GFR (slope)	GFR slope = 3.7 (3.1-4.3) mL/min vs 4.2 (3.6-4.9) mL/min
MDRD (1994 overall with long-term follow-up)[17, 18]	840 Aged 18-70, with GFR 13-55 mL/min/1.73^2, MAP <125 mmHg & dietary protein intake >0.9g/kg body weight/d.	LOW BP target (MAP ≤92 mmHg; equivalent to BP <125/75 mmHg if ≤60 years and ≤98 mmHg if >60 years) AND usual, low or very low protein diet (n=432)	USUAL BP target (MAP ≤107 mmHg; equivalent to BP of 140/90 mmHg if ≤60 years and ≤113 mmHg if >60 years) AND usual, low or very low protein diet (n=408)	52	268 vs 286	HR 0.68 (0.57-0.82) P>0.001	ESRD or all-cause mortality 312 vs 312	HR 0.77 (0.65-0.91) P=0.0024

Study Name Acronym (year)	Study population	Active Treatment	Control	Mean age (years)	No of ESRD events (active vs control)	ESRD analysis (95% CI)	Clinical composite and No of events for analysis	Clinical composite Analysis (95% CI)
REIN 2 (2005)[19]	338, Aged 18-70, non-diabetic nephropathy & persistent proteinuria, not received ACEi for >6 weeks. Proteinuria 1-3 g/d included if CrCl <45 mL/min/1.73m^2; proteinuria >3 g/d included if CrCl <70 mL/min/1.73 m^2.	INTENSIVE BP control to <130/80 mmHg (n=169)	CONVENTIONAL BP control (DBP <90 mmHg irrespective of SBP) (n=169)	53.9	38 vs 34	HR 1.00 (0.61-1.64) P=0.99	GFR decline mL/min/1.73 m^2/month	0.22 (IQR 0.06-0.55) vs 0.24 (IQR 0.0001-0.56) P=0.62
Schrier (2002)[20]	75 Aged 20-60, with ADPKD and left ventricular hypertrophy, CrCl >30 ml/min/1.73m^2	INTENSIVE BP control <120/80 mmHg (n=41)	STANDARD BP control 135-140/85-90 mmHg (n=34)	41	5 vs 3	NA	NA	NA
SPRINT (2016)[21] (CKD; eGFR <60 mL/min/1.73m^2)	2,646 with CKD, hypertension and 1 other CV risk factor	INTENSIVE BP control SBP <120 mmHg (n=1,330)	CONVENTIONAL BP control SBP <140 mmHg (n=1,316)	67.9	6 vs 10	HR 0.57 (0.19-1.54) P=0.27	eGFR reduction ≥50%, dialysis, or transplantation 14 vs 15	HR 0.89 (0.42-1.87) P=0.76
SPRINT (2016)[21] (eGFR ≥60 mL/min/1.73m^2)	6,677 without CKD, and hypertension and 1 other CV risk factor	INTENSIVE BP control SBP <120 mmHg (n= 3,332)	CONVENTIONAL BP control - SBP <140 mmHg (n = 3,345)	67.9	NA	NA	≥30% reduction in eGFR to <60 ml/min/1.73m^2 137 vs 27	HR 3.49 (2.44-5.10) P<0.001
Toto (1995)[22]	87 Aged 25-73, without diabetes with hypertension (DBP 95 mmHg), and GFR 70 m/min/1.73m^2	STRICT BP control DBP 65-80 mmHg (n=42)	CONVENTIONAL BP control DBP 85-95 mmHg (n=35)	55.7	7 vs 2	NA	Rate of decline in GFR ml/min/1.73m^2/year	-0.31±0.45 (-1.73 to +1.95) vs -0.05±0.50 (-2.32 to +3.09) P>0.25

AASK = African American Study of Kidney Disease and Hypertension; ESCAPE = Effect of Strict Blood Pressure Control and ACE Inhibition on the Progression of Chronic Renal Failure in Paediatric Patients; MDRD = Modification of Diet in Renal Disease; REIN = Ramipril Efficacy in Nephropathy; SPRINT = Systolic Blood Pressure Intervention Trial.

ACEi = angiotensin converting enzyme inhibitor; ADPKD = autosomal dominant polycystic kidney disease; CKD = chronic kidney disease; CrCl = creatinine clearance; CV = cardiovascular; CVD = cardiovascular disease; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; GFR = glomerular filtration rate; ESRD = end-stage renal disease; HR = hazard ratio; IDDM = insulin-dependent diabetes mellitus; MAP = mean arterial pressure; RR(R) = relative risk reduction; SBP = systolic blood pressure; SCr = serum creatinine; UACR = urine albumin:creatinine ratio.

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Appendix 2:

UK HARP-III Baseline results publication and Supplementary data:

Judge PK, Haynes R, Herrington WG, Storey BC, Staplin N, Bethel A, Bowman L, Brunskill N, Cockwell P, Dayanandan R, Hill M, Kalra PA, McMurray JJ, Taal M, Wheeler DC, Landray MJ, Baigent C. Randomized multicentre pilot study of sacubitril/valsartan versus irbesartan in patients with chronic kidney disease: United Kingdom Heart and Renal Protection (HARP)-III - rationale, trial design and baseline data. Nephrol Dial Transplant 2017;32(12):2043-2051.

Nephrol Dial Transplant (2016) 0: 1–9
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Original Article

Randomized multicentre pilot study of sacubitril/valsartan versus irbesartan in patients with chronic kidney disease: United Kingdom Heart and Renal Protection (HARP)- III—rationale, trial design and baseline data

UK HARP-III Collaborative Group

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ABSTRACT

Background. Patients with chronic kidney disease (CKD) are at risk of progression to end-stage renal disease and cardiovascular disease. Data from other populations and animal experiments suggest that neprilysin inhibition (which augments the natriuretic peptide system) may reduce these risks, but clinical trials among patients with CKD are required to test this hypothesis.

Methods. UK Heart and Renal Protection III (HARP-III) is a multicentre, double-blind, randomized controlled trial comparing sacubitril/valsartan 97/103 mg two times daily (an angiotensin receptor–neprilysin inhibitor) with irbesartan 300 mg one time daily among 414 patients with CKD. Patients ≥18 years of age with an estimated glomerular filtration rate (eGFR) of ≥45 but <60 mL/min/1.73 m^2 and urine albumin:creatinine ratio (uACR) >20 mg/mmol or eGFR ≥20 but <45 mL/min/1.73 m^2 (regardless of uACR) were invited to be screened. Following a 4- to 7-week pre-randomization single-blind placebo run-in phase (during which any current renin–angiotensin system inhibitors were stopped), willing and eligible participants were randomly assigned either sacubitril/valsartan or irbesartan and followed-up for 12 months. The primary aim was to compare the effects of sacubitril/valsartan and irbesartan on measured GFR after 12 months of therapy. Important secondary outcomes include effects on albuminuria, change in eGFR over time and the safety and tolerability of sacubitril/valsartan in CKD.

Results. Between November 2014 and January 2016, 620 patients attended a screening visit and 566 (91%) entered the pre-randomization run-in phase. Of these, 414 (73%) participants were randomized (mean age 63 years; 72% male). The mean eGFR was 34.0 mL/min/1.73 m^2 and the median uACR was 58.5 mg/mmol.

Conclusions. UK HARP-III will provide important information on the short-term effects of sacubitril/valsartan on renal function, tolerability and safety among patients with CKD.

Keywords: cardiovascular disease, chronic kidney disease, neprilysin, progression

INTRODUCTION

Chronic kidney disease (CKD) affects between 2 and 17% of the general population (depending on the country) [1, 2] and is associated with increased risks of progression to end-stage renal disease (ESRD) and morbidity and mortality from cardiovascular disease (CVD) [3, 4]. Renin–angiotensin system (RAS) inhibitors [angiotensin-converting enzyme inhibitors (ACEis) and angiotensin receptor blockers (ARBs)] have been shown to reduce the risk of ESRD in patients with proteinuric CKD [5–8], but despite such treatments, patients remain at significant risk of progression to ESRD and CVD.

The natriuretic peptide (NP) system is a neurohormonal system that has a variety of potentially beneficial functions, including natriuresis, diuresis, vasodilatation and counterregulation of RAS [9, 10]. The NP system can be augmented by inhibiting the main enzyme responsible for degrading NPs, namely neprilysin [or neutral endopeptidase (NEP)] [10]. NEP is a membrane-bound zinc-containing metalloproteinase [11]

that also degrades other peptides, including angiotensin II, bradykinin, endothelin and substance P [12]. However, isolated NEP inhibition (NEPi) leads to reflex RAS activation, and inhibits angiotensin II breakdown (counteracting any potentially beneficial effects) and therefore NEPi must be combined with RAS inhibition.

As NEPi and ACEi both inhibit bradykinin degradation, their combination is associated with substantially elevated bradykinin levels that cause unacceptable rates of angioedema [13]. ARBs do not inhibit bradykinin degradation and can be safely combined with NEPi [creating a new class of drugs called angiotensin receptor–neprilysin inhibitors (ARNis)]. Sacubitril/valsartan (previously known as LCZ696) is the first drug in this new class, combining valsartan with sacubitril [(AHU377) a prodrug that is metabolized via esterases to the active NEPi sacubitrilat (LBQ657)]. Sacubitril/valsartan 97/103 mg provides equivalent plasma concentrations of valsartan as oral valsartan 160 mg [14].

In a 5/6 nephrectomy model, treatment with combined NEP/RAS inhibition was associated with greater reductions in proteinuria and glomerulosclerosis compared with RAS inhibition alone [15, 16]. Micropuncture studies also demonstrated NEPi led to greater reductions in capillary glomerular pressure [15]. Among patients with heart failure, trials comparing sacubitril/valsartan with either ACEi or ARB have suggested that the estimated glomerular filtration rate (eGFR) of patients allocated sacubitril/valsartan declined less than those assigned ACEi or ARB [17, 18]. Sacubitril/valsartan also reduced blood pressure more than equivalent doses of valsartan in trials among patients with elevated blood pressure [19]. Trials in heart failure populations suggest NEPi might increase albuminuria [18, 20], but this effect was not observed in patients with hypertension [19] and baseline albuminuria was very low in all these trials. Overall, these data raise the hypothesis that treatment with an ARNi may be superior to either ACEi or ARB alone in slowing the progression of CKD.

The United Kingdom (UK) Heart and Renal Protection III (HARP-III) trial (ISRCTN11958993) was designed to provide information on the short-term efficacy (in terms of effect on renal function), tolerability and safety of sacubitril/valsartan among patients with CKD. The trial will also assess the effects of sacubitril/valsartan on albuminuria, blood pressure and biomarkers of kidney and cardiac damage.

MATERIALS AND METHODS

Study design

UK HARP-III is a double-blind, multicentre, randomized controlled trial comparing sacubitril/valsartan 97/103 mg two times daily versus irbesartan 300 mg one time daily among at least 400 participants ≥18 years of age with stages 3 and 4 CKD. Irbesartan 300 mg was selected as the comparator, as it has been shown to reduce the risk of ESRD among patients with diabetic kidney disease and is licensed for the treatment of proteinuric CKD [6, 21]. Participants were randomly allocated to receive sacubitril/valsartan or irbesartan and will be followed up for 1 year (Figure 1). The primary aim of UK HARP-III is to assess the effect of sacubitril/valsartan 97/103 mg two times daily versus irbesartan 300 mg one time daily on measured glomerular filtration rate (mGFR) at 12 months. Important secondary outcomes include the effect on urine albumin:creatinine ratio (uACR) and eGFR. All the secondary and tertiary assessments are shown in Figure 2 and further details are available in the data analysis plan (see Supplementary data). A summary of substantial amendments to the protocol is provided in the Supplementary data.

Eligibility

To fulfil the inclusion criteria, patients need to be ≥18 years of age and have either an eGFR ≥45 but <60 mL/min/1.73 m^2 with a uACR >20 mg/mmol or eGFR ≥20 but <45 mL/min/

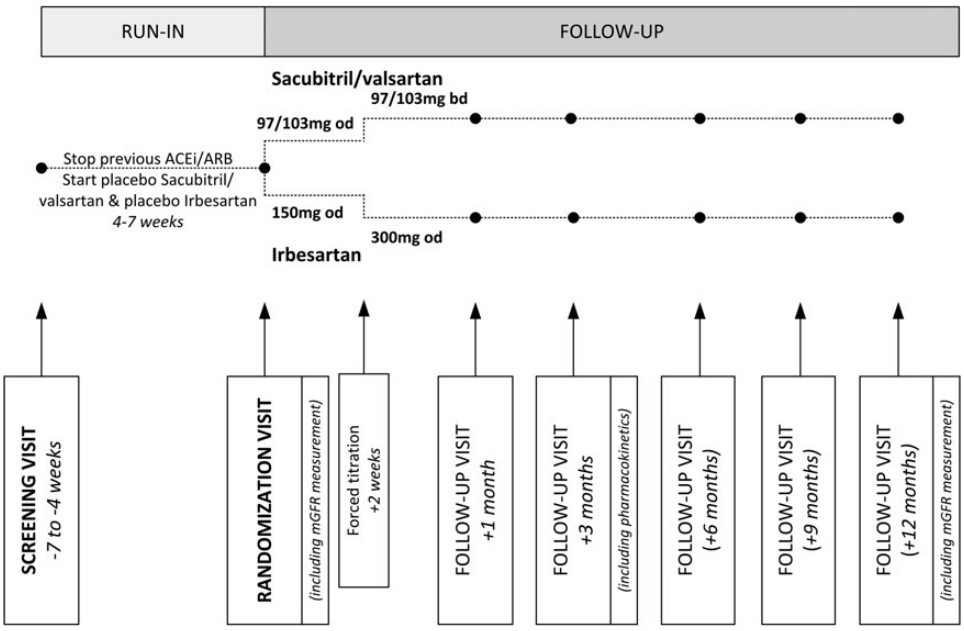

FIGURE 1: UK HARP-III trial design.

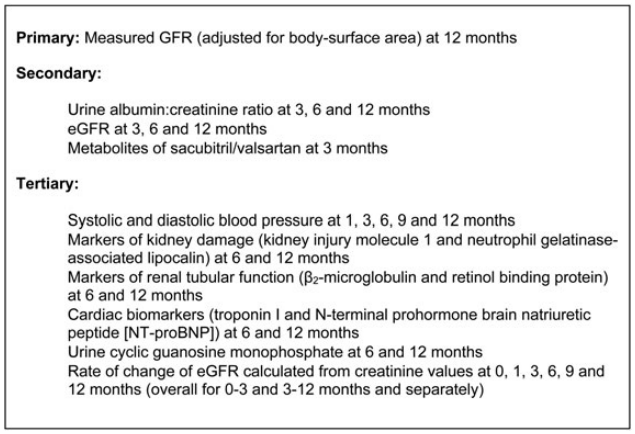

FIGURE 2: UK HARP-III trial outcomes.

1.73 m^2 (regardless of uACR). The exclusion criteria were designed to identify patients for whom the safety of sacubitril/valsartan or irbesartan may have been a concern. The full eligibility criteria are shown in Figure 3.

Study enrolment and randomization

Identification and invitation. After relevant ethics [Nottingham Research Ethics Committee 2 (13/EM/0434)] and regulatory approvals had been obtained, sites were established in UK renal units. Site staff identified potentially eligible patients from hospital electronic databases, mailed these individuals an invitation letter and a copy of the patient information sheet and called them ~1 week later to discuss the trial in more detail, answer any questions they might have and to see whether they were interested in participating. Those individuals interested in participating were invited to attend a screening visit.

Screening. At the screening visit, eligibility was assessed and written informed consent was obtained from eligible individuals. All data were recorded directly into a bespoke Internet-based electronic case report form system. Relevant details of their medical history (including primary renal diagnosis, presence of diabetes mellitus and prior CVD) were recorded by trained research nurses and their height, weight and blood pressure were measured. Blood pressure was measured and recorded three times using an Omron M6 automated digital sphygmomanometer after sitting for at least 5 minutes. Willing and eligible patients entered the pre-randomization run-in phase. Samples of blood and urine were sent to the local hospital laboratory for confirmation of eligibility. If the results were considered inaccurate (e.g. haemolysed sample) by the local study staff the samples could be repeated once, but if the results did not confirm eligibility the participant was withdrawn from the run-in phase.

Pre-randomization run-in. The aims of the pre-randomization run-in phase were (i) to 'wash out' any ACEi prior to introduction of NEPi, (ii) to allow a comparison of the acute effects of the study treatments on GFR and (iii) to reduce the rate of post-randomization discontinuation of study treatment and to produce a consequent improvement in the trial's statistical sensitivity [22]. Following the screening visit, any current ACEi and/or ARB that the participant was taking was stopped and the participant entered the 4- to 7-week single-blind pre-randomization run-in phase, during which they were asked to take one placebo sacubitril/valsartan tablet and one placebo irbesartan capsule once daily. If elevated blood pressure became a concern during the run-in phase, local investigators were advised to titrate up or start additional anti-hypertensive medications, but to avoid an ACEi, ARB or direct renin inhibitor (DRI). The choice of additional anti-hypertensive therapy remained at the discretion of the responsible clinician. Participants could withdraw from the trial for any reason during this run-in phase. Participants who did not withdraw returned 4–7 weeks later and had their GFR measured and attended a randomization visit. GFR was measured using a standard ^{51}Cr-EDTA technique, although if this was not available at the site, other methods (^{99m}Tc-DTPA or iohexol) could be used with the agreement of the coordinating centre. In willing participants, a 24-hour collection of urine for albumin and sodium quantification was also obtained.

Randomization visit. Participants were not eligible for randomization if the mean of their second and third measurements of systolic blood pressure was <110 mmHg (or <130 mmHg with symptoms of hypotension) or if they reported an adverse event they believed to be related to their run-in treatment. Participants who remained willing and eligible were then randomly allocated in a 1:1 ratio to receive either sacubitril/valsartan or irbesartan. Participants were randomized by an Internet-based system using a minimization algorithm to ensure balance of important predictors of renal progression, including age, sex, systolic blood pressure, eGFR, uACR and the presence or absence of diabetes mellitus.

At the randomization visit, run-in treatment was collected and willing and eligible participants were issued two bottles of study treatments: one containing sacubitril/valsartan 97/103 mg or placebo tablets and the other containing irbesartan 150 mg or placebo capsules (therefore a double-dummy technique to protect blinding). Participants were initially instructed to take one tablet and one capsule daily in the morning (i.e. either sacubitril/valsartan 97/103 mg plus placebo irbesartan or placebo sacubitril/valsartan plus irbesartan 150 mg). Blood and urine samples were collected for the local analysis of creatinine, electrolytes, liver function tests and uACR and others were prepared for central analysis (Table 1).

Post-randomization follow-up

Randomization is now complete and all participants are in follow-up. In order to check potassium and renal function after starting study treatment, participants attend their study clinic or local primary care physician at 2 weeks after randomization for a blood sample. If these results are satisfactory, study treatments are increased to either sacubitril/valsartan 97/103 mg two times daily plus two capsules of placebo irbesartan one time daily or one tablet of placebo sacubitril/valsartan two times daily plus irbesartan 300 mg one time daily.

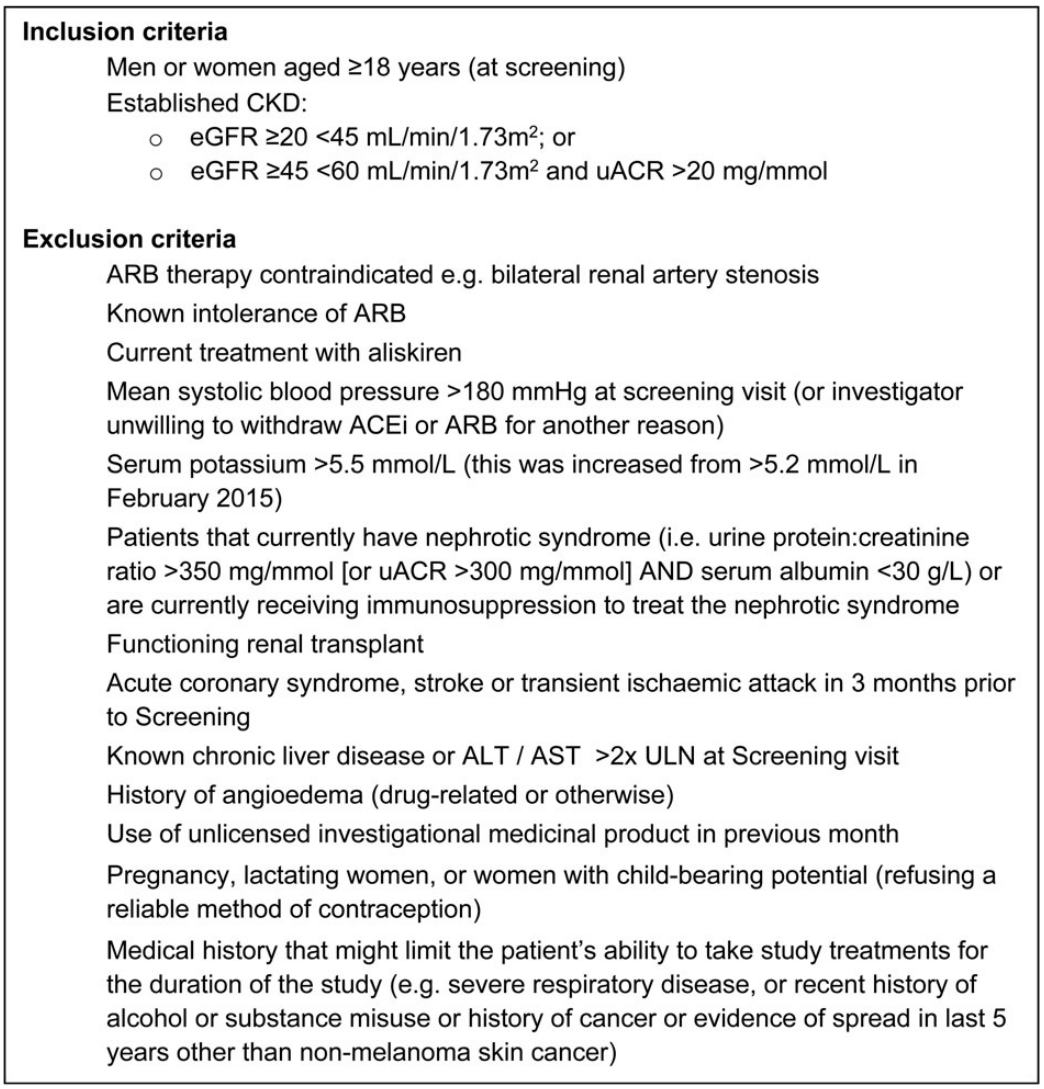

FIGURE 3: Inclusion and exclusion criteria. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

Follow-up assessments. Study follow-up visits are scheduled at 1, 3, 6, 9 and 12 months after randomization. At all visits, study staff systematically seek the information on all serious adverse events, any non-serious adverse events considered by participants to be related to study treatment; and on symptoms of hepatitis. Compliance with study treatment is assessed and participants unable to tolerate the maximum dose of study treatments are encouraged to continue on the lower dose of study drug (i.e. sacubitril/valsartan 97/103 mg or irbesartan 150 mg daily) for the remainder of the trial. If relevant, a reason for discontinuation or dose reduction is recorded. Participants prescribed contraindicated medications (ACEi, ARB or DRI) have their randomized treatment stopped. Weight and blood pressure are measured (three times after sitting for at least 5 minutes) at all visits. In both treatment groups, blood pressure is to be controlled according to the Kidney Disease: Improving Global Outcomes guidelines [23], with the initiation and choice of additional anti-hypertensive treatment being at the discretion of the responsible clinician. Within the 2 weeks before their 12-month visit participants have their second GFR measurement (using the same method as at baseline). Copies of results of both measurements of GFR are sent to the coordinating centre so the results entered by site staff can be verified by clinical study staff blind to the treatment allocation.

Biological samples and safety monitoring. At each follow-up visit, blood and urine samples are sent to the local hospital laboratory for creatinine, electrolytes, liver function tests (bilirubin, alanine or aspartate transaminase and alkaline phosphatase) and uACR. In addition, at the 3-, 6- and 12-month visits, samples are also taken for central analysis. EDTA samples are centrifuged and the plasma aliquoted into Cryovials, which are stored locally (with Cryovials of urine) at or below −20°C prior to transfer to the central laboratory in Oxford, UK, where they are stored at −80°C. The main plasma analytes measured at the central laboratory are creatinine, cardiac and inflammatory biomarkers and the urine analytes include albumin and markers of tubular damage and function [including kidney injury molecule 1, neutrophil gelatinase-associated lipocalin, β2-microglobulin and retinol binding protein; Table 1]. Participants are asked not to take their morning dose of study treatment on the day of their 3-month visit (at

Table 1. Planned central laboratory blood and urine analyses

Analyte	Time point			
	Randomization	3 months	6 months	12 months
EDTA plasma samples				
Creatinine	■	■	■	■
Albumin	■		■	■
Troponin-I	■		■	■
NT-proBNP	■		■	■
CRP	■		■	■
IL-6	■		■	■
Urine samples				
Albumin:creatinine ratio	■	■	■	■
KIM-1	■		■	■
NGAL	■		■	■
cGMP	■		■	■
β-2-microglobulin	■		■	■
Retinol binding protein	■		■	■

NT-proBNP, N-terminal prohormone brain natriuretic peptide; CRP, C-reactive protein; IL-6, interleukin 6; KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin; cGMP, cyclic guanosine monophosphate.

this visit only) and the date and time of the last dose is recorded, as these samples are to be used for pharmacokinetic analyses.

The results of local samples are entered into the trial database once available and reviewed daily by a trained clinician at the coordinating centre. If the potassium is >5.5 mmol/L, alanine or aspartate transaminase >2× the upper limit of normal or if the eGFR has fallen >25% from the previous value, then the trial protocol provides advice on further tests and study treatment (see Supplementary data).

Monitoring

Prior to starting recruitment, study staff received training in the study procedures and the web-based data collection system at the coordinating centre. Recruitment rates, adherence to trial procedures and completeness of follow-up data are monitored closely by staff at the coordinating centre. All sites have at least one on-site monitoring visit, with further visits as indicated by the results of central monitoring of the data. An independent data monitoring committee (see Supplementary data) regularly reviews unblinded interim analyses of all relevant data.

Statistical considerations

Sample size. The chief aim of this study is to compare mGFR between the two treatment groups at the final follow-up visit. Analysis of covariance (ANCOVA) compares mean follow-up mGFR between treatment groups after adjustment for baseline mGFR [24]. Assuming a between-person standard deviation (SD) in mGFR of 15 mL/min/1.73 m^2 and a correlation between an individual's baseline and follow-up mGFR of 0.8, randomization of 400 participants will provide at least 80% power (at 2 P = 0.05) to detect a difference in mGFR at the final follow-up (adjusted for baseline values) of 3 mL/min/1.73 m^2 (the chosen minimum clinically meaningful difference), even if 15% of participants discontinue allocated study treatment [20].

Statistical analysis. All analyses will involve comparing outcomes during the scheduled treatment period among all those participants allocated at randomization to receive sacubitril/valsartan 97/103 mg two times daily versus all those allocated to receive irbesartan 300 mg one time daily [i.e. intention-to-treat (ITT) analyses] [25, 26]. Comparisons of continuous outcomes (including the primary outcome) between the allocated treatment arms will be performed using ANCOVA adjusted for each patient's value at baseline [27]. If continuous outcomes are not normally distributed, then appropriate transformations (e.g. log transformation) will be made. Multiple imputation techniques will be used to account for any missing data in the primary and secondary outcomes [28]. Further details are provided in the data analysis plan (see Supplementary data).

RESULTS

Study sites were established in 24 renal units in the UK. Between November 2014 and January 2016 a total of 620 patients attended the study screening visits and 566 (91%) entered the pre-randomization run-in (Figure 4).

Pre-randomization run-in

A total of 138 participants withdrew from the pre-randomization run-in before attending a randomization visit (Table 2A). The most common medical reason for withdrawal from run-in was that the results from blood and urine samples taken at the screening visit did not confirm the participant's eligibility (Table 2A). Adverse events were uncommon and four participants were withdrawn because of a serious adverse event (myocardial infarction, septic shock and two cases of pneumonia).

In addition, 14 individuals attended a randomization visit but were not eligible to be randomized: the most common reason for this was their blood pressure being too low (Table 2B). Overall, 152 (27%) of the 566 individuals who entered the pre-randomization single-blind placebo run-in phase were not subsequently randomized.

Baseline characteristics of randomized participants

A total of 414 people were randomized (Figure 4). The mean age was 63 (SD 14) years and 298 (72%) were male (Table 3). The mean systolic blood pressure was 146 (SD 16) mmHg at randomization (i.e. after 4–7 weeks of withdrawal of any prior ACEi or ARB). Based on results from the local laboratories, the mean eGFR was 34.0 (SD 10.6) mL/min/1.73 m^2 and the median uACR was 58.5 (interquartile range 12.5–156.3) mg/mmol. Central laboratory assays will be conducted at the end of the study. About half of randomized participants had either glomerular [111 (27%)] or diabetic [83 (20%)] kidney disease and 165 (40%) patients reported diabetes mellitus at baseline. The median 5-year risk of ESRD (calculated using a validated risk calculator [29] was 16.5%, and 62% of participants had a 5-year risk >10%.

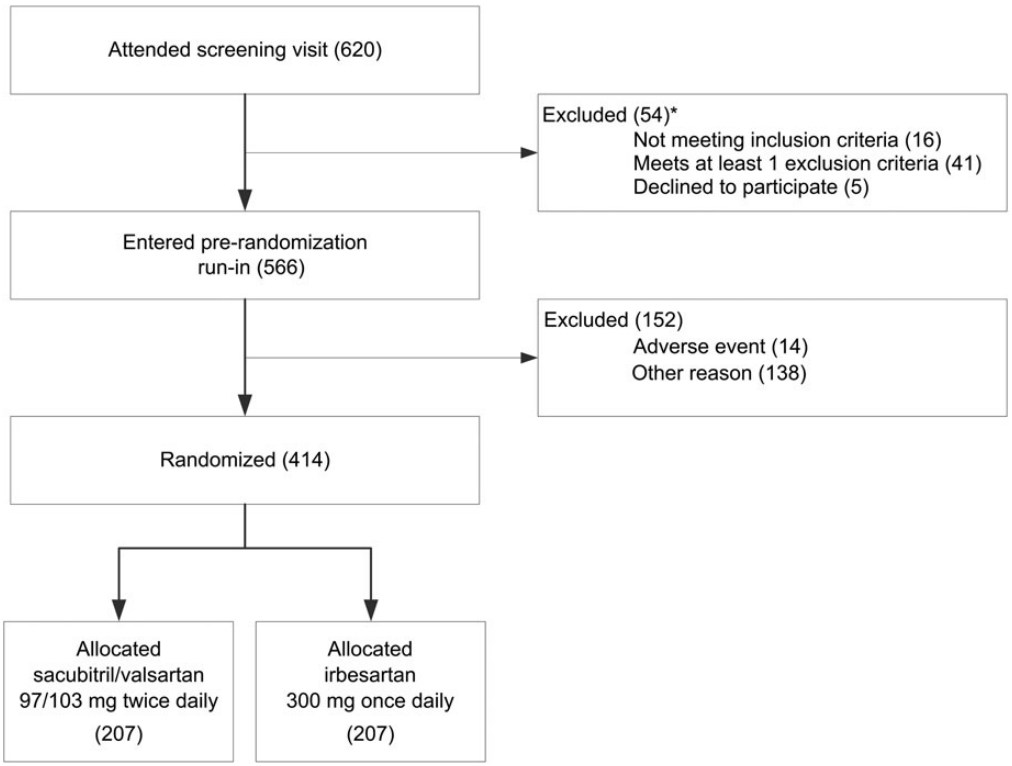

FIGURE 4: Trial profile: flow of participants through the trial. *Indicates that participants may have more than one reason.

Table 2. Reasons for (A) withdrawal during run-in and (B) ineligibility at a randomization visit

(A)	*n* (%)
Number entering run-in	566
Adverse event	
Serious adverse event	4 (3)
Non-serious adverse reaction	7 (5)
Other reason	
Ineligible on laboratory results sent at screening visit	59 (43)
Participant wishes	16 (12)
Medical advice	13 (9)
Other non-medical reason	39 (28)
Total withdrawn during Run-in	138 (100)
(B)	
Number attending randomization visit	428
Adverse event	
Serious adverse event	0 (0)
Non-serious adverse reaction	3 (21)
Other reason	
Blood pressure too low	9 (64)
Other	2 (14)
Total ineligible at randomization visit	14 (100)

DISCUSSION

The UK HARP-III trial has recruited 414 participants with CKD and will provide information on the short-term effects of sacubitril/valsartan on the change in kidney function (using mGFR) and the tolerability and safety of the drug compared with irbesartan in people with CKD. The trial will also provide information on the effects of sacubitril/valsartan on albuminuria, blood pressure and other biomarkers of both kidney and cardiac function. These results are important because sacubitril/valsartan has now entered routine clinical practice as a treatment for heart failure with reduced ejection fraction (HFrEF) [30], and many of these patients also have CKD. Moreover, NEPi has the potential to be a useful treatment for CKD itself.

Large randomized trials of interventions to slow the progression of CKD are required since currently available treatments do not prevent ESRD in all patients with CKD. Although ACEis and ARBs reduce the risk of progression of proteinuric diabetic and non-diabetic kidney disease, their effect (like most medical treatments) is moderate. For example, in proteinuric diabetic kidney disease, irbesartan reduced the risk of ESRD, doubling of creatinine or death from any cause by 20% compared with placebo {hazard ratio [HR] 0.80 [95% confidence interval (CI) 0.66–0.97]; P = 0.02}, but this composite outcome still occurred in nearly one-third of those allocated irbesartan (and 14% reached ESRD) during the mean 2.6 years of follow-up [6]. Other strategies to reduce the risk of renal progression have either been ineffective, hazardous or both [31–33]. Neprilysin inhibition appears to be effective in rat models of CKD [15, 16, 34], but these are poorly predictive of efficacy in humans [35, 36]. In addition, sacubitril/valsartan has been shown to increase albuminuria in trials among patients with heart failure (who typically have very low baseline albuminuria) [18, 20]. NPs (particularly atrial NP) cause afferent arteriolar vasodilatation [37, 38] that may lead to increased intraglomerular pressure and hyperfiltration, which would be detrimental to the kidney. However, NEPi also disturbs degradation of other

Table 3. Baseline characteristics of UK HARP-III participants

Baseline characteristic	All participants ($n = 414$)
Age (years)	63 ± 14
<50	73 (18)
≥50–70	196 (47)
≥70	145 (35)
Gender	
Male	298 (72)
Female	116 (28)
Ethnicity	
White	377 (91)
Black	7 (2)
South Asian	18 (4)
Other	12 (3)
Prior disease	
Coronary heart disease	55 (13)
Cerebrovascular disease	31 (7)
Peripheral arterial disease	44 (11)
Heart failure	17 (4)
Diabetes	165 (40)
Systolic blood pressure (mmHg)	146 ± 16
<140	149 (36)
≥140–160	180 (43)
≥160	85 (21)
Diastolic blood pressure (mmHg)	81 ± 11
<80	191 (46)
≥80–90	133 (32)
≥90	90 (22)
Body mass index (kg/m^2)	30.6 ± 6.2
<25	68 (16)
≥25–30	147 (36)
≥30	195 (47)
Not available	4 (1)
Medication	
Antiplatelet therapy	138 (33)
Oral anticoagulant	28 (7)
Diuretic	164 (40)
Calcium channel blocker	207 (50)
β-blocker	112 (27)
α-blocker	112 (27)
LDL-lowering agent	263 (64)
Prior use of ACEi or ARB	
Yes	339 (82)
No	75 (18)
CKD-EPI eGFR (mL/min/1.73 m^2)	34.0 ± 10.6
<30	169 (41)
≥30–45	176 (43)
≥45	64 (15)
Not available	5 (1)
Urine albumin:creatinine ratio (mg/mmol)	58.5 (12.5–156.3)
<3	48 (12)
≥3–< 30	88 (21)
≥30	251 (61)
Not available	27 (7)
Primary renal diagnosis	
Glomerular disease	111 (27)
Tubulointerstitial disease	50 (12)
Diabetic kidney disease	83 (20)
Hypertensive/renovascular disease	42 (10)
Other systemic diseases affecting the kidneys	3 (1)
Familial/hereditary nephropathies	43 (10)
Miscellaneous renal disorders	9 (2)
Unknown	73 (18)

Recorded at randomization visit unless otherwise stated. Values are given as *n* (%), mean ± SD or median (interquartile range).
CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; LDL, low-density lipoprotein.

vasoactive peptides, so the net effect of NEPi on glomerular haemodynamics is uncertain, and in rat models at least, it appears to be favourable [15, 16, 34]. NPs may alter glomerular permeability and/or tubular reabsorption of protein, which may lead to albuminuria without hyperfiltration, the consequences of which are uncertain. UK HARP-III is the first trial of NEPi in humans with CKD and the measurements of GFR, albuminuria and other markers of kidney function and damage will help to resolve these uncertainties.

Most patients with CKD do not progress to ESRD [39], but are at high risk of CVD [4]. Lowering low-density lipoprotein cholesterol has been shown to clearly reduce the risk of atherosclerotic vascular disease in CKD [40]. However, as renal function declines, the pattern of CVD changes from atherosclerotic disease (i.e. myocardial infarction, ischaemic stroke) to non-atherosclerotic disease (characterized by arteriosclerosis and structural heart disease, which manifests clinically similarly to heart failure, with a high incidence of sudden cardiac death) [4, 41–43], but effective treatments for non-atherosclerotic disease are not yet available. Lowering blood pressure in patients with CKD appears to reduce the risk of a wide variety of cardiovascular events, but residual risk remains [44]. The similarities in the manifestation of non-atherosclerotic disease observed in CKD and heart failure suggest that treatments that are effective in heart failure may well also be effective at reducing cardiovascular risk among patients with CKD. In the Prospective Comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) trial, sacubitril/valsartan reduced the risk of cardiovascular mortality or hospitalization for heart failure by 20% [HR 0.80 (95% CI 0.73–0.93) $P < 0.001$] compared with enalapril, with similar effects observed among participants with and without CKD at baseline [45]. These data suggest that NEPi would be an ideal candidate to test among patients with CKD. Nevertheless, most patients with CKD have a normal ejection fraction [41, 43], and treatments that improve outcomes in HFrEF do not necessarily improve outcomes in patients with heart failure with preserved ejection fraction [46, 47], so direct evidence is needed. NEPi has improved cardiac biomarkers (e.g. troponin, N-terminal prohormone brain NP in trials in heart failure [18, 48], so the effects of NEPi on these cardiac biomarkers in people with CKD will also be of interest.

NEPi has the potential to improve both renal and cardiovascular outcomes among patients with CKD. The UK HARP-III trial will provide important information on the efficacy, safety and tolerability of sacubitril/valsartan in people with CKD. Results are anticipated in 2017.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTEREST STATEMENT

CTSU has a staff policy of not accepting honoraria or other payments from the pharmaceutical industry, expect for the reimbursement of costs to participate in scientific meetings (www.ctsu.ox.ac.uk).

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APPENDIX

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Randomized multicentre pilot study of sabubitril/valsartan versus irbesartan in patients with chronic kidney disease: UK Heart and Renal Protection (HARP)-III.

Rationale, trial design and baseline data

SUPPLEMENTARY APPENDIX

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Randomized multicentre pilot study of sacubitril/valsartan versus irbesartan in patients with chronic kidney disease:

UK Heart and Renal Protection (HARP)-III

Data Analysis Plan

Contents

1. Introduction

The purpose of this Data Analysis Plan is to provide a clear definition of the main randomized analyses to be reported in the primary report of the UK HARP-III trial results, before unblinding of the treatment allocation. The nature of further analyses and the content of subsequent publications cannot be specified in detail but, where appropriate, the general analytical approach is set out.

2. Outcomes in UK HARP-III

2.1 Primary outcome

The primary outcome is mean measured glomerular filtration rate (mGFR; adjusted for body-surface area) at 12 months. Glomerular filtration rate (GFR) will be measured using a ^{51}Cr-EDTA or other approved technique.

2.2 Secondary outcomes

The secondary outcomes are:

- Mean urine albumin:creatinine ratio (uACR) at 3, 6 and 12 months from centrally analysed urine samples
- Estimated GFR (eGFR) at 3, 6 and 12 months from centrally analysed plasma samples using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) formula
- Metabolites of sacubitril/valsartan measured in blood samples taken at 3 months

2.3 Tertiary outcomes

The tertiary outcomes are:

- Systolic and diastolic blood pressure (mmHg) at 1, 3, 6, 9 and 12 months
- Markers of renal damage (kidney injury molecule-1 [KIM-1] and neutrophil gelatinase-associated lipocalin [NGAL]) at 6 and 12 months

- Markers of renal tubular function (β_2-microglobulin and retinol binding protein) at 6 and 12 months
- Urine cyclic guanosine monophosphate (cGMP) excretion at 6 and 12 months
- Cardiac biomarkers (troponin I and N-terminal prohormone brain natriuretic peptide [NT-proBNP]) at 6 and 12 months
- Rate of change of eGFR calculated from creatinine values at Randomization, 1, 3, 6, 9 and 12 months (overall, and separately for 0-3 months [ie, Randomization, 1 and 3 month values] and 3-12 months [ie, 3, 6, 9 and 12 month values]) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (see section 4.4.). Where values from the central laboratory are available (randomization, 3, 6 and 12 months) these will be used, but local values will be used at 1 and 9 months

3. Baseline characteristics

In order to assess balance of baseline characteristics between randomized arms, the following variables recorded at randomization will be presented for each of the sacubitril/valsartan and irbesartan groups:

- Age
- Sex
- Past medical history (prior diabetes mellitus, prior vascular disease)
- Blood pressure (systolic and diastolic separately)
- Body mass index
- Baseline mGFR
- Baseline albuminuria
- Baseline 24 hour urinary sodium excretion (top versus bottom half)
- Current/recent medication (including any renin-angiotensin system [RAS] blockade)
- Cause of kidney disease (glomerular, tubulointerstitial, diabetic, hypertensive/renovascular, other systemic diseases, familial/hereditary, other known causes and unknown cause)

4. Comparisons of sacubitril/valsartan versus irbesartan

All comparisons will involve comparing outcomes during the scheduled treatment period among *all* those participants allocated at randomization to receive sacubitril/valsartan 97/103 mg twice daily versus all those allocated to receive irbesartan 300mg once daily (i.e. "intention-to-treat" [ITT] analyses).[1, 2]

4.1 Primary assessment

Mean mGFR at 12 months will be compared between all participants allocated sacubitril/valsartan and all participants allocated irbesartan. Estimates will be made by analysis of covariance (ANCOVA) after adjustment for each participant's baseline mGFR. Missing or implausible mGFR values will be handled as described in section 6.1.3.

4.2 Secondary assessments of the primary outcome

Mean mGFR at 12 months (the primary outcome) among sacubitril/valsartan-allocated and irbesartan-allocated participants will be compared separately by the following baseline characteristics:

- Age (≤60; >60 years)
- Sex (Female, Male)
- History of diabetes mellitus (Yes, No)
- History of vascular disease (Yes, No)
- Systolic blood pressure (≤140; >140 mmHg)
- Diastolic blood pressure (≤80; >80 mmHg)
- Body mass index (top versus bottom half)
- Baseline mGFR (≤45; >45 mL/min/1.73m^2)
- Baseline uACR (≤30 mg/mmol; >30 mg/mmol)
- Baseline 24 hour urinary sodium excretion (top versus bottom half, ignoring participants with missing values)
- Use of RAS blockade at screening (Yes, No)
- Cause of kidney disease (in categories as in Section 3)

4.3 Assessment of secondary outcomes

Mean uACR (or an appropriate transformation of uACR) and eGFR at 3, 6 and 12 months will be compared between all participants allocated sacubitril/valsartan and all participants allocated irbesartan using ANCOVA to adjust for baseline values. This will be done both separately at the three follow-up time points and overall (using the mean of the 3, 6 and 12 month values).

Pharmacokinetic analyses will also be conducted using measurements of sacubitril/valsartan metabolite trough concentrations measured at 3 months after randomization (see section 6.1.4).

4.4 Assessment of tertiary outcomes

Mean values of tertiary outcomes (or mean of an appropriate transformation of the outcome) at 6 and 12 months will be compared between all participants allocated sacubitril/valsartan versus all patients allocated irbesartan. ANCOVA will be used to estimate the mean value adjusted for the baseline value. As systolic and diastolic blood pressure are also measured at 1, 3 and 9 months, analyses of these outcomes will also be done separately for the 1, 3 and 9 month follow-up visits, as well as overall (using a weighted average of the 1, 3, 6, 9 and 12 month values).

For each participant, linear regression will be used to estimate the rate of change in eGFR from the available creatinine values. The validity of making such a linearity assumption will be assessed by examining the residuals. The participants with the most poorly fitting slopes (defined as participants with the mean deviation from their own fitted slope in the top 1% of the distribution [of mean deviations across all participants]) will be excluded. The mean rate of change in eGFR will then be compared between participants allocated sacubitril/valsartan versus patients allocated irbesartan.

Any other comparisons of the tertiary outcomes will also be between all participants allocated sacubitril/valsartan versus all participants allocated irbesartan but will be exploratory only (with due allowance in the interpretation for multiplicity and the retrospective nature of such analyses).

5. Safety and tolerability outcomes

The safety and tolerability of sacubitril/valsartan will be assessed from the following information. The analyses of these data are described in section 6.1.5.

5.1 Serious adverse events (SAEs)

All SAEs, regardless of whether the SAE is considered related to study treatment, will be recorded, and subdivided by outcome (fatal/non-fatal). The numbers and proportions of participants with SAEs in each group (sacubitril/valsartan and irbesartan) will be described. Particular SAEs of interest include:

- Angioedema
- Hypotension
- Need for dialysis (recorded on electronic case report form)

5.2 Reported reasons for stopping study treatment

All reasons for stopping treatment will be recorded and listed in relevant categories by treatment allocation. All adverse events, including non-serious adverse events, that cause participants to discontinue study treatment will be recorded and grouped by treatment allocation according to MedDRA version 14.0 primary system organ class. The numbers and proportions of participants in each treatment group with non-serious adverse events and serious adverse events that result in discontinuation of study treatment will be described. In particular, discontinuation of study treatment due to the following reasons is of interest:

- Angioedema
- Hypotension
- Hyperkalaemia
- Deterioration in renal function
- Abnormal liver function tests (alanine aminotransferase [ALT] and aspartate aminotransferase [AST])

5.3 Biochemical safety data

The biochemical safety data collected will include kidney and liver related outcomes, in particular:

- Potassium (≥5.5 <6.0; ≥6.0 <6.5; ≥6.5 mmol/L)
- 25% reduction in eGFR since randomization
- ALT/AST >10x upper limit of normal (ULN)
- ALT/AST >3x ULN and bilirubin ≥2x ULN
- Consecutive ALT/AST >3x ULN (ie, two consecutive measurements at least 3 days apart)

6. Details of analyses

6.1 Methods of analysis

6.1.1 ANCOVA

Comparisons of continuous outcomes (eg, mGFR, uACR, systolic and diastolic blood pressure and any other biomarkers or physical measurements) between the allocated treatment arms will be performed using ANCOVA adjusted for each patient's value at randomization.[3] If continuous outcomes are not normally distributed then appropriate transformations (e.g. log transformation) will be made.

6.1.2 Repeated measures

Where more than one follow-up value of a biomarker is available, comparisons of the mean values of the biomarker will be conducted at each follow-up time using ANCOVA adjusted for each participant's baseline value of the biomarker. In addition, a weighted average of all the follow-up values (with weights proportional to the amount of time between visits) will be calculated for each participant and the mean values compared using ANCOVA adjusted for each participant's baseline value.

6.1.3 Imputation of missing data

All analyses will be done according to the intention-to-treat principle and hence, where missing, primary and secondary outcome data will be imputed. For each of the continuous outcomes (eg, mGFR, uACR) missing post-randomization results will be imputed using multiple imputation, using 20 imputed data sets, with results across imputations being combined using the methods of Rubin.[4] The imputation procedure will take into consideration each participant's key baseline characteristics (listed in section 3), treatment allocation and any intermediate follow-up values of the biomarker, where available. For patients who commence chronic dialysis during the study and for whom it is not possible to measure GFR at study end, a value of 0 will be imputed for the final mGFR. Values will be imputed for patients who die prior to their second mGFR. The results from these analyses will be compared with those from equivalent "complete-case" analyses, but primary emphasis will be placed on the results after multiple imputation. All multiple imputation analyses will be implemented using the multiple imputation procedure in SAS version 9.3 (SAS Institute, Cary NC), using the expectation-maximization algorithm (which assumes a multivariate normal distribution) to impute values. For any continuous variables with missing baseline values, the mean among those with observed values will be imputed.

6.1.3.1 Participants who refused consent for 12 months follow-up

A small number of participants who had been randomized before the protocol was amended to extend follow-up from 6 to 12 months refused consent for 12 months follow-up. These participants will have a mGFR performed at 6 months. Multiple imputation will be used to impute 12 month mGFR values for these participants, including all available information on GFR at 6 months in the imputation model.

6.1.3.2 Implausible mGFR values

Technical issues can cause GFR measurements to give spurious results, but this is typically not apparent until after the participant has already been randomized and started their randomized allocation (or stopped taking study treatment at the end of the trial). The differences between each mGFR value and its corresponding creatinine-based eGFR value (ie, the value based on a blood sample taken at the same timepoint as the mGFR) will be calculated, and the distribution of these differences inspected before any unblinded analyses

are performed. Based on this inspection a threshold will be determined (eg, 95 or 99% centiles) such that any values that fall outside this threshold are ignored. Multiple imputation will be used to handle any missing values of mGFR generated.

6.1.4 Pharmacokinetic assessments

The objective of pharmacokinetic analyses is to quantify the determinants of plasma concentrations of metabolites of sacubitril/valsartan (including LBQ657 [sacubitrilat], the active metabolite of sacubitril [AHU377]).

A single trough plasma sample for pharmacokinetic analysis is collected at the 3 month visit (including time since last drug dosage). This will be sent to a third party laboratory (WuXi AppTec, Shanghai, China) for measurement of sacubitril, valsartan and LBQ657. Participants will be included in analyses from these analyses if they were allocated and taking sacubitril/valsartan at the 3 month visit and the plasma sample was taken between 10 to 16 hours after the last dose. Plasma concentrations of sacubitril, valsartan and LBQ657 will be tabulated by baseline mGFR (unadjusted for body surface area). In addition, appropriate population pharmacokinetic modelling techniques will be used to identify the determinants of the plasma concentration of each metabolite. The variables to be assessed will include baseline mGFR (unadjusted for body surface area), time since last dose, albuminuria, age, sex, body surface area and weight.

6.1.5 Safety analyses

All participants randomized to sacubitril/valsartan will be compared with all participants randomized to irbesartan, regardless of whether a participant received all, some or none of their allocated treatment (ie, ITT).[1, 2] A participant may contribute to more than one assessment if they have events of more than one type (e.g. non-fatal hypotension followed by angioedema).

For reasons for stopping and safety biochemical outcomes, the effect of allocated treatment on the number of randomized participants with at least 1 event will be compared using standard tests for differences in proportions.

For the time-to-event analyses of adverse events, the effect of allocated treatment will be evaluated using survival analytic methods on the time to first event during the entire study period. For each outcome, the log-rank method will be used to estimate the average event rate ratio comparing all those allocated sacubitril/valsartan with all those allocated irbesartan.[2] Estimates of event rate ratios will be shown with 95% confidence intervals and their associated log-rank p-values. In all analyses, two-sided p-values (2P) <0.05 will be considered statistically significant (after any adjustment for multiple testing [see section 6.2]).

6.2 Allowance for multiplicity of comparisons

The primary outcome will be assessed without adjustment for multiplicity. For secondary and particularly the tertiary and exploratory analyses, allowance in their interpretation will be made for multiple hypothesis testing,[1, 2] taking into account the nature of events (including timing, duration and severity) and evidence from other studies. In addition to the pre-specified comparisons, many other analyses will be performed with due allowance for their exploratory and, perhaps, data-dependent nature. Conventionally, two-sided P-values <0.05 are often described as “significant”. But, the larger the number of events on which a comparison is based and the more extreme the P-value (or, analogously, the further the confidence interval is from zero) after any allowance has been made for the nature of the particular comparison (i.e. primary, secondary or tertiary; pre-specified or exploratory), the more reliable the comparison and, hence, the more definite any finding will be considered.

6.3 Tests for heterogeneity

When a number of different subgroups are considered, chance alone may lead to there being no apparent effect in several subgroups in which the effect of treatment really is about the same as is observed overall. In such circumstances, “lack of direct evidence of benefit” is not good “evidence of lack of benefit”, and clearly significant overall results would provide strong indirect evidence of benefit in some small subgroups where the results, considered in isolation, are not conventionally significant (or, even, perhaps, slightly adverse).[1, 2] Hence, unless the proportional effect in some specific subcategory is clearly different from that observed overall, the effect in that subcategory is likely to be best estimated indirectly by applying the proportional effect observed among all patients in the trial to the absolute risk of the event observed among control patients in that category.

Tests for heterogeneity of the proportional effect observed in subgroups will be used (with allowance for multiple comparisons) to determine whether the proportional effects in specific subcategories are clearly different from the overall effect.[1, 2] If, however, three or more patient categories can be arranged in some meaningful order (e.g. age at randomization: <50, ≥50<60, ≥60) then assessment of any trend will be made. For subgroups based on continuous variables (e.g. blood pressure, kidney function), approximate similar sized divisions (such as by tertiles) may be used, using natural breaks to define categories (e.g. systolic blood pressure <140 mmHg rather than <138.7 mmHg). These breaks will be defined exactly prior to any unblinding of results.

7. References

1. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. I. Introduction and design. *Brit J Cancer* 1976; 34: 585-612.

2. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. *Brit J Cancer* 1977; 35: 1-39.

3. Vickers AJ, Altman DG. Statistics notes: Analysing controlled trials with baseline and follow up measurements. *Brit Med J* 2001; 323: 1123-4.

4. Rubin D. Multiple imputation for non-response in surveys. New York: John Wiley; 1987.

Safety monitoring procedures

1. Abnormal potassium

a. Potassium >5.5 <6.0 mmol/L

- Confirm potassium in non-haemolyzed sample
- Inform Local Lead Investigator (LLI) within 72 hours
- Dietary advice to avoid potassium-rich food and drink
- Review non-study medications (including over-the-counter medications) and stop potassium-sparing medications if possible
- Consider checking for acidosis and correcting if present
- Repeat potassium measurement within 7 days
    - If potassium remains >5.5 <6.0 mmol/L, discuss frequency of potassium monitoring with LLI (and coordinating centre if required)
    - If potassium ≤5.5 mmol/L, return to routine follow-up

b. Potassium ≥6.0 mmol/L

- Confirm potassium in non-haemolyzed sample
- Inform LLI immediately
- Discontinue study treatments
- Dietary advice to avoid potassium-rich food and drink
- Review non-study medications (including over-the-counter medications) and stop potassium-sparing medications if possible
- Consider checking for acidosis and correcting if present
- Repeat potassium measurement within no more than 3 days (ideally 1-2 days if potassium >6.5 mmol/L)
    - If <6.0 mmol/L, restart study treatment and discuss frequency of potassium monitoring with LLI (and coordinating centre if required)
    - If ≥6.0 mmol/L remain off all study treatment permanently

2. Unexpected changes in estimated glomerular filtration rate (eGFR)

If the eGFR falls by more than 25% between study visits (in particular, during the titration period in the first month after randomization) the LLI should be informed so that alternative causes of deterioration may be investigated (e.g. hypovolaemia, obstruction, non-study medications). The eGFR should be re-measured within 7 days (fewer days if a larger fall) in case of measurement error. If necessary the dose of study treatment can be modified, ideally after discussion with the coordinating centre.

3. Abnormal alanine aminotransferase (ALT) or aspartate aminotransferase (AST)

ALT or AST (xULN)	Symptoms[1]/bilirubin	Action	Follow-up monitoring
≤3	Not relevant	None	Repeat at next study visit
>3 ≤5	Absent and Bilirubin <2x ULN	Inform LLI Repeat in 1 week: investigate for cause[**] if still >3	At LLI's discretion
>5 ≤8	Absent and Bilirubin <2x ULN	Inform LLI Repeat in 2-4 days: investigate for cause[**] if still >3	Repeat ALT/AST, ALP and bilirubin until resolution (frequency at LLI's discretion) If >5 for >2 weeks, stop study treatment
>8	Absent and Bilirubin <2x ULN	Inform LLI Repeat in 2-4 days: if still >8, stop study treatment. Investigate for cause[**]	Repeat ALT/AST, ALP and bilirubin until resolution (frequency at LLI's discretion) If >5 for >2 weeks, stop study treatment
>3	Present or Bilirubin ≥2x ULN	Inform LLI Stop study treatment Investigate for cause[**]	Repeat ALT/AST, ALP and bilirubin until resolution (frequency at LLI's discretion) Consider restart if alternative cause found

ULN = upper limit of normal; LLI = local lead investigator;

[1] Symptoms of liver disease e.g. malaise, fatigue, abdominal pain, nausea, vomiting, jaundice
[**] Careful history of alcohol, non-study medications, travel, diet, hepatobiliary ultrasound, viral and autoimmune serology

Substantial amendments to the study protocol

Version	Date	Original text	Amended text	Rationale
5.0	04/09/2014	Version approved by ethics committee and regulatory agency prior to recruitment beginning		
5.1	19/11/2014		First morning void urine samples will be collected at each study visit for local and central analysis	First morning urine samples reduce intra-individual variability compared with random urine samples
6.0	09/03/2015	Inclusion criteria: eGFR ≥20 <60 mL/min/1.73m^2 and urine albumin:creatinine ratio >20 mg/mmol Exclusion criteria: Serum potassium > 5.2 mmol/L Systolic BP <130 mmHg at Randomization	Inclusion criteria: eGFR ≥20 <45 mL/min/1.73m^2; or eGFR ≥45 <60 mL/min/1.73m^2 and urine albumin:creatinine ratio >20 mg/mmol Exclusion criteria: Serum potassium > 5.5 mmol/L Systolic BP <110 mmHg (or <130 mmHg with symptoms of orthostatic hypotension) at Randomization	To facilitate recruitment and avoid unnecessary exclusion of participants
7.0	11/05/2015	Follow-up duration 6 months	Follow-up duration 12 months	New data from heart failure population suggested that the full effects on renal function may take at least 9 months to emerge with sacubitril/valsartan[1]
8.0	25/01/2016	Original sample size 360 participants (based on assumption that 10% might discontinue study treatment)	Sample size increased to at least 400 participants	To allow for up to 15% of participants to discontinue study treatment

1. Voors AA, Gori M, Liu LC, et al. Renal effects of the angiotensin receptor neprilysin inhibitor LCZ696 in patients with heart failure and preserved ejection fraction. *Eur J Heart Fail* 2015; 17: 510-7.

Appendix 3:

UK HARP-III trial study poster.

UK HARP-III

UK Heart and Renal Protection Study

Do you have Chronic Kidney Disease and are you interested in research?

Some people with chronic kidney disease will develop end stage renal disease requiring long-term dialysis or transplantation. UK HARP-III is a national study being coordinated by the University of Oxford which is investigating a new treatment to slow the progression of kidney disease and the development of circulatory problems such as heart attacks and strokes.

If you have kidney disease (but are not on dialysis or have a transplant), then UK HARP-III could be suitable for you.

LCZ696

LCZ696 is a new drug with two actions: one half is an "angiotensin receptor blocker" which is a drug commonly used in heart and kidney disease. (Such drugs include valsartan, losartan and irbesartan.)

The other half of the drug prevents the breakdown of certain proteins ("natriuretic peptides") in the bloodstream. There is some evidence to suggest that preventing the breakdown of these proteins may have beneficial effects on protecting the kidney. This might slow the rate of progression of kidney disease and delay the need for dialysis or transplantation. There may also be beneficial effects on the heart and blood vessels.

UK HARP-III is comparing LCZ696 with irbesartan (a commonly used angiotensin receptor blocker) to investigate whether LCZ696 might improve outcomes for people with chronic kidney disease.

Where can I find out more about UK HARP-III

Further details are available on our website: www.harp3trial.org

Or you can speak to a member of the study team:

What do I do if I am interested in taking part?

Please contact the study team who will be able to tell you if you are suitable for the study.

Thank you for your interest.

Appendix 4:

UK HARP-III trial participant information leaflet.

UK Heart And Renal Protection (UK HARP-III) Trial

LCZ696 in chronic kidney disease: a pilot study

An invitation to join a research study

We would like to invite you to take part in an important study about kidney disease. The study could provide doctors with much better information about how to reduce the illness worsening in thousands of patients around the world.

Please take the time to read this information leaflet before making a decision about whether or not to join this study. It is important that you know why the research is being done, and what it might mean for you. Feel free to discuss this information with family and friends. Please contact us if there is anything more you would like to know.

What is the purpose of the study?

The study is called the UK HARP-III trial. It is investigating whether a new drug (LCZ696) has the potential to protect kidneys better than current standard treatment.

Chronic kidney disease affects about 1 in 10 adults. The illness can worsen over time. This means that some people eventually need to have dialysis or a kidney transplant.

There are treatments that can slow the rate of kidney decline. However, despite such treatment some people still need transplantation or dialysis. Two commonly-used treatments are:

- "ACE inhibitors" (you may be familiar with them as their drug names end with the letters "-pril". And,
- Angiotensin receptor blockers (the drug names end in "-sartan")

LCZ696 is a new treatment which has two actions: one half of the drug is the same as an angiotensin receptor blocker (valsartan). The other half of the drug is a "neprilysin inhibitor" which prevents the breakdown of certain proteins in the blood. Blocking their breakdown might slow the progression of kidney damage and delay the need for dialysis and transplant. These drugs may also benefit the heart and blood circulation.

Why have I been invited?

Your medical records suggest that you may be suitable because you have chronic kidney disease. We have informed your kidney consultant about the study and they are happy for us to discuss it with you. If you take part your GP will be informed.

Do I have to take part?

No. Participation is entirely up to you. If you agree to take part we will ask you to sign a form to show that you have consented. You are free to withdraw at any time, without giving a reason. This will not affect the standard of care you receive.

Who is running and who is funding the study?

UK HARP-III is being led by experienced medical scientists at the University of Oxford who carried out the important Study of Heart and Renal Protection (SHARP). This study showed the benefits of lowering "bad" cholesterol in people with chronic kidney disease and resulted in changes to medical practice around the world. Treatment for this study is provided free by Novartis (a pharmaceutical company), which also contributes to the cost of running of the study, by a grant to the University of Oxford. The results will be analysed by scientists at the University of Oxford independently of Novartis.

What will happen to me if I take part?

You will need to participate in UK HARP-III for about 13 months. You will be asked to attend about seven hospital appointments, some of which may coincide with your routine renal outpatient appointments. In addition, we will measure your kidney function very precisely on two occasions during the study.

Getting started At your first visit to the HARP-III clinic a trained researcher (usually a nurse) will ask you about your medical history. They will explain the study to you and you will be given plenty of opportunity to ask questions. The researcher will take your blood pressure and a sample of blood and urine. If you are interested in the study, you will be asked to sign a form agreeing to take part. We will write to your GP about your participation in the study. You will then be provided with a supply of the study tablets and asked to take two a day. This visit will take about 45 minutes.

You may be asked to stop some of your current blood pressure treatment (because the study treatment will replace them). Over the course of the next few weeks you will have the chance to try out the study tablets. This will allow you and the UK HARP-III doctors and nurses to be sure the routine of taking these particular tablets agree with you. You will be given a container and asked to collect a sample of your urine on the morning of your next visit and bring it with you to the study clinic. Towards the end of this period you may also be asked to collect your urine for 24 hours, but this optional and you can still participate even if you don't want to do this.

After 4 to 7 weeks After at least 4 weeks of taking the study tablets you will be asked to attend a second appointment to see if you would like to continue. We will measure your kidney function very precisely (see below). Your blood pressure will be checked and we will ask for another blood sample. Your height and weight will also be recorded at this visit. If you have had no problems with the study treatments during the first few weeks and are happy to continue, you will be asked to commit to the study for another 12 months. This visit will take about 30 minutes.

At this appointment your treatment for the rest of the study will be decided "at random" (like the toss of a coin). You will be given two drugs (one, an active treatment and the other a 'dummy' placebo). These will be either active LCZ696 and placebo irbesartan, or placebo LCZ696 and active irbesartan. Irbesartan is an angiotensin receptor blocker and is commonly used to treat kidney disease. The initial dose is one tablet of each treatment once daily.

You have as much chance of receiving LCZ696, as you do of receiving the standard treatment, irbesartan. You will not know which treatment you receive, nor will your GP or the UK HARP-III staff.

However, this information would be made available to your doctor and other medical staff if this was medically necessary.

Further visits You will be asked to have a blood test after about two weeks to check your potassium level. This can be done at your GP surgery or your renal clinic. The dose of your study treatment will then increase to the full dose (two tablets of active treatment and two tablets of placebo once daily).

You will be asked to attend five further appointments (about 1, 3, 6, 9 and 12 months later) to see how you are getting on. You will be asked to bring a urine sample collected on the morning of each of these visits to the clinic (in a container provided at the previous visit). Your blood pressure will be checked and a blood sample will be taken at each visit. At the final visit you will also have a second precise measurement of your kidney function. Each visit will last about 30 minutes.

In the unlikely event that your blood test results are of concern (for example, if your potassium level was high) you may be asked to attend an extra visit. We would repeat the blood test and further checks would be done. With regular check-ups from the UK HARP-III specialist nursing team, you can be assured of the best possible follow-up care and attention. If any problems emerge for you while you are on the study, your consultant and GP will be informed.

Blood and urine samples

The blood and urine samples that you provide will be tested locally to check that the study treatments are not having any adverse effects. We need about 4 teaspoons of blood on each occasion. Some of the samples will also be sent to the central laboratory in Oxford University. This allows us to see whether the effects of the treatments vary between different types of people taking part. We will also look to see if the treatments affect other markers of kidney function.

We will also ask for permission to store your blood and urine samples long-term. These samples will not have your name on. This will help with other kidney studies and research into other diseases.

Measuring kidney function

In routine clinical practice, your doctors estimate kidney function by looking at the level of a substance called creatinine in the blood. This is sufficient for clinical purposes, but in the UK HARP-III study we need to measure kidney function more precisely. This involves having an injection of a very small amount of a substance (which in some hospitals may be radioactive) and then a number of blood samples in the 4-5 hours that follow (this may take longer at certain hospitals). This allows us to measure how quickly your kidneys remove this substance from your blood. This test is routinely used in the NHS when kidney function needs to be measured precisely. If used, the amount of radioactivity is very small (equivalent to about ten days of normal background radiation or less in the UK), so represents a negligible risk to your health.

Travel expenses

We are happy to reimburse reasonable expenses for travelling to your UK HARP-III appointments. Please make sure you ask about this at the clinic.

What will I have to do?

For UK HARP-III to produce the best results, it is important that people stay in the study for its duration if possible. You will need to attend the UK HARP-III clinic seven times during the 13-14 months of the study. Extra appointments can also be arranged if you are worried about the study tablets. However, you can withdraw from the study at any time.

You will be asked to take either a drug called irbesartan or the new treatment, LCZ696. Scientists do not know which treatment is best. You may be asked to stop some of your current medications because the study treatment will replace them. We will discuss this with you at your appointment.

We will ask you to provide blood and urine samples and to give permission for them to be stored for future tests. We will also ask about your health. Your blood pressure will be measured at every visit. At some visits extra measurements will be taken, including your height and weight.

What are the benefits of taking part in this study?

You may be helping yourself, but you will most certainly be helping doctors and scientists improve treatment for people who have chronic kidney disease and who may be at risk of needing dialysis or a transplant. If successful, results from this study will help to design a larger trial of LCZ696 which could reliably show whether LCZ696 is better than current treatment in slowing the progression of chronic kidney disease.

Are there any risks?

Most treatments have side-effects which some people may experience and others do not. If you do experience any side effects while on the UK HARP-III study they will be noted, so that scientists can learn from you. You can withdraw from the study if you wish.

- **Irbesartan** is generally very well-tolerated. It has been tested in thousands of people and is taken by hundreds of thousands of people worldwide. It lowers blood pressure so it can cause dizziness. Other side-effects include nausea, muscle pain and fatigue. Like all "angiotensin receptor blockers" it can raise potassium levels in the blood and you will be monitored for this.
- **LCZ696** is an unlicensed drug and is being tested in this study. Over 8,000 people have taken LCZ696 in other trials and it is generally well-tolerated. It also lowers blood pressure so can cause dizziness and fatigue. Rarely it may cause swelling of the mouth and face (angioedema), but it does not appear to do this more frequently than "ACE inhibitors" which are a very commonly used medication in people with chronic kidney disease. It is very important that you do not take LCZ696 with an ACE inhibitor (e.g. ramipril, lisinopril). The treatment can raise potassium levels in the blood and you will be monitored for this. One patient who received LCZ696 had an allergic reaction which included abnormal liver function tests. At this stage, scientists cannot rule out the possibility of there being side effects (such as diarrhoea or muscle pains), or effects on other blood tests.

  A large trial of LCZ696 in patients with heart problems recently showed that LCZ696 reduced admissions to hospital with heart problems or dying of circulatory problems. In this population the treatment was well-tolerated and there were no concerning safety problems. UK HARP-III is testing LCZ696 in a different group of patients i.e. people with chronic kidney disease.

  Throughout the study you would be carefully monitored by our nursing team for possible side effects. At every visit, the study staff would discuss any new information about the drug with you.

There is nothing to suggest that stopping the tablets will cause you harm. If you do experience side effects, you may choose, or be advised by your doctor, to stop the tablets provided by the study.
If you do experience unexpected symptoms after joining the study you can contact your UK HARP-III nurse, or a study doctor on **Freefone 0800 585323** (available 24 hours a day, 7 days a week).

What are the other possible disadvantages of taking part?

The study includes two precise measurements of your kidney function which may involve an injection of a small amount of radioactive material. The dose of radioactivity is small (equivalent to ten days of natural background radiation or less in the UK) and poses a negligible risk to health.

Before participating you should check whether doing so will affect any insurance that you have and seek advice if necessary.

For women Irbesartan should not be taken by pregnant or breast-feeding women. The effects of LCZ696 on pregnancy and the unborn child and breast-feeding are not known therefore such individuals would not be eligible to participate in the study. Women who could become pregnant must use effective and reliable methods of contraception[1] (listed in the footnote below) during the course of this study and for 7 days after the end of the study (i.e. after stopping study treatment).

If you become pregnant during the trial (or wish to do so), you should tell your study nurse or doctor immediately so appropriate action can been discussed.

What happens when the study stops?

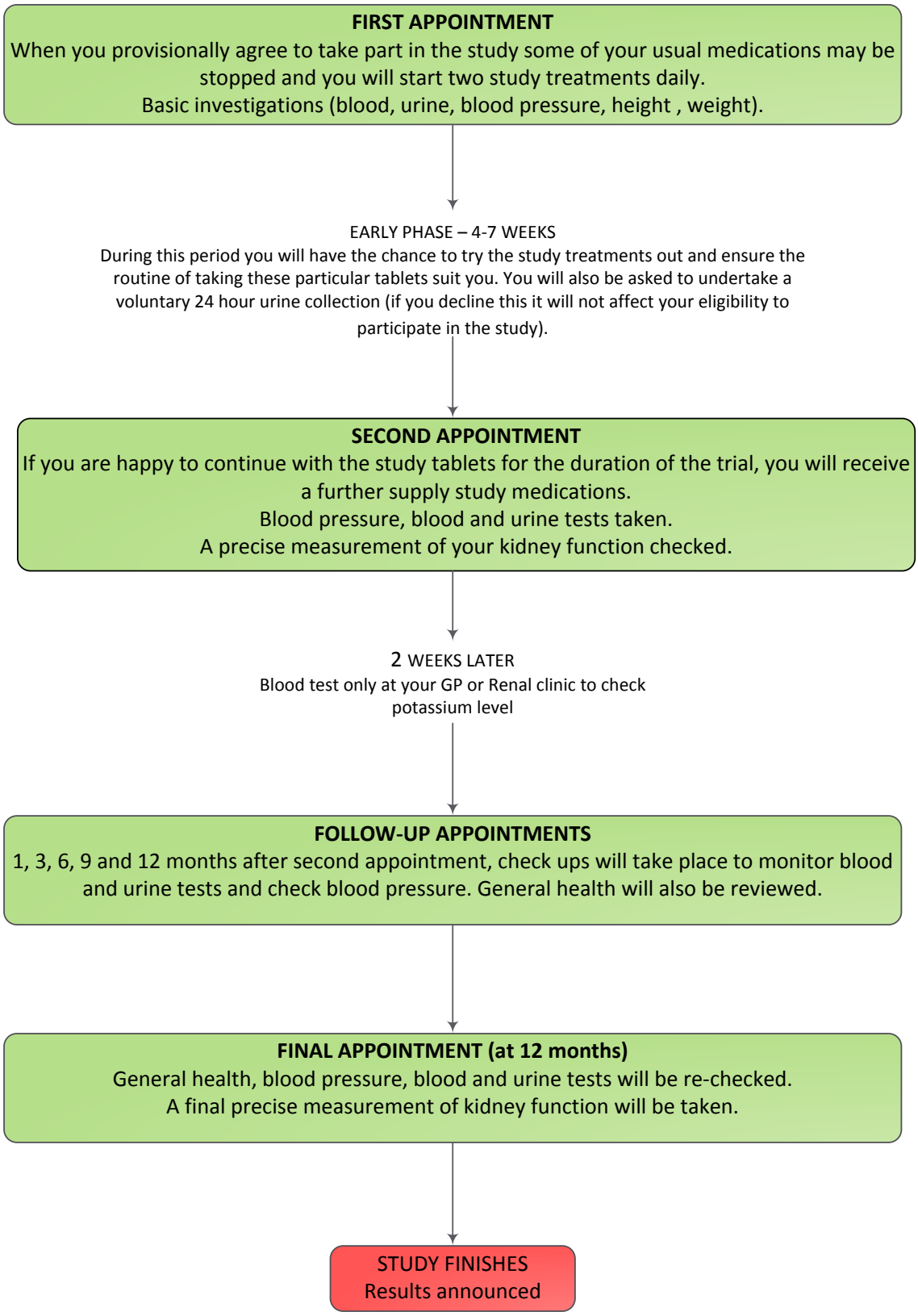
You and your doctors will be informed of the study results when they become available. LCZ696 does not have a license in the UK currently so it will not be available once the study is complete. However this study will help design a larger trial of LCZ696 in people with chronic kidney disease which could lead to it becoming available. At the end of the study you will go back to any treatment that you stopped. Your doctor will advise you about this.

What if there is a problem?

If you have any concerns about any possible side-effects of treatment or any complaint about the way you have been dealt with during the study, please call the study team on **Freefone 0800 585323**. More detailed information is given in Part 2.

[1] Highly effective methods of contraception include; injectables, the combined oral contraceptive pill (if taking the combined pill, you must have been taking a stable dose for at least 3 months before entering the study), an intrauterine device, vasectomised partner, or true sexual abstinence (this does not include periodic abstinence measures such as calendar, ovulation, symptothermal or post-ovulation methods).

STUDY TIMETABLE



Participants stop study medications and study clinic visits. Pre-study usual medications maybe started by your doctor. Continued follow-up with renal clinic as planned.

Part 2: Further details for patients who want them

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatments that are being studied. If this happens, your study doctor will tell you about it and discuss whether you want to or should continue in the study. If you decide not to carry on, your doctor will make the necessary arrangements. On receiving new information your doctor might consider it to be in your best interests to stop the study treatments. They will explain the reasons and arrange for your care to continue. If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

What if I don't want to carry on with the study?

You are free to withdraw at any time. If you wish to discontinue your study treatment we would advise you to do this in consultation with your doctor so they can arrange other suitable treatment. We would still like to see you in the study clinic, even if you are not taking the study tablets, so that we can ensure the study's results are as reliable as possible.

For women Women who could become pregnant must continue to use effective methods of contraception for 7 days after stopping study treatment (or the end of the study.)

What if there is a problem?

You retain all the usual rights of an NHS patient.

The University of Oxford has appropriate insurance in place in the unlikely event that you suffer any harm as a direct consequence of your participation in this trial.

If you have a concern about any aspect of the study you can speak with the researchers. They can be contacted on a 24-hour Freefone number: 0800 585323 or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on 01865 572224 or the head of CTRG, email ctrg@admin.ox.ac.uk. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the Patient Advice and Liaison Service at your hospital.

Will my taking part be kept confidential?

Yes, absolutely. If you accept this invitation, your basic contact details will be recorded so your first appointment can be made (and these details could only be seen by your local research team and staff working in the coordinating centre in Oxford). Nurse monitoring staff from the coordinating centre in Oxford may occasionally ask your permission to be present during your clinic visit to ensure procedures are being properly followed. The coordinating centre will seek information from your doctors and from NHS and other central registries about any serious illnesses that may occur. This requires your name, date of birth and NHS number. All information received will be used, in confidence, only for medical research purposes and for routine regulatory and audit purposes. Responsible members of the University of Oxford or the host NHS Trust may be given access to data for monitoring and/or audit of the study to ensure we are complying with regulations. Anonymised data collected during the study may be sent to Novartis (the company funding the trial).

Blood samples are sent to a laboratory at the University of Oxford for analysis. They are identified by a unique number linked in the computer to other study information. In the laboratory they are not linked to your name. The information used for scientific analysis will not include any details that

identify you. Any information from stored samples will not be provided to you, your doctors, or anybody else. In particular, having these samples stored and subsequently tested would not affect your ability to obtain insurance.

What will happen to the results of the research study?

It is intended to present the results at a major medical conference and publish them in an appropriate medical journal. No patient will be individually identified in any report or publication.

How is this study organised?

Scientists and doctors consider the questions being asked by UK HARP-III to be important because they could improve treatment for people who have chronic kidney disease. Scientists at the University of Oxford are coordinating the study with the collaboration of many doctors and nurses from around the country. The study design has been reviewed and agreed by independent Research Ethics Committee (Nottingham 2 Research Ethics Committee, reference 13/EM/0434). These committees check whether the health question being asked is important enough to warrant a study, and that the study is being carried out in an independent, honest and professional manner.

An independent committee also watches over the study and keeps an eye on results. This committee could stop the study early if important new evidence emerged that had an impact on the need for the study to continue.

Independent studies such as UK HARP-III are costly to run. Treatment for the study is provided free by Novartis, which also contributes to the cost of running of the study, by a grant to the University of Oxford. However, UK HARP-III is conducted independently of Novartis and the study information will be collected, analysed and published independently of the source of funding.

Thank you

Thank you for your interest in this study. Our aim is to make your participation an interesting and worthwhile experience, while helping us and others to improve the treatment of people who have chronic kidney disease.

Questions about the study should be directed to the coordinating centre in Oxford

By phone:

24-hour Freefone service: **0800 585323**

By post:

UK HARP-III, Clinical Trial Service Unit (CTSU), Richard Doll Building, University of Oxford, Roosevelt Drive, OXFORD, OX3 7LF

By email:

harp3@ctsu.ox.ac.uk

Or visit our website:

www.harp3trial.org

Appendix 5:

UK HARP-III trial study treatment information leaflet.

Other medications

At each visit the study nurse will check that any other medications you are taking are compatible with both LCZ696 and irbesartan. However, it is important that any doctor treating you (including your GP) knows that you are taking part in UK HARP-III so any prescription they give you will also be compatible. They may call **Freefone 0800 585323** if they have any queries.

Throughout the study duration, **Angiotensin Converting Enzyme (ACE) inhibitors**, **Angiotensin Receptor Blockers (ARBs)** and **Direct renin inhibitors** such as aliskiren (Rasilez), should not be taken with your UK HARP-III study treatment at any time. Examples of these medications are listed below.

ACE inhibitors	ARBs
Lisinopril (Zestril, Carace Plus, Zestoretic)	**Losartan** potassium (Cozaar, Cozaar-Comp)
Ramipril (Tritace, Triapin)	**Valsartan** (Diovan, Co-Diovan, Exforge)
Perindopril (Coversyl, Coversyl Arginine Plus)	**Irbesartan** (Aprovel, CoAprovel
Enalapril (Innovace, Innozide)	**Candesartan** cilexetil (Amias)
Captopril (Captoten)	**Telmisartan** (Micardis, Micardis Plus)
Cilazapril (Vascace)	**Olmesartan** (Olmetec, Sevikar, Sevikar HCT, Olmetec Plus)
Fosinopril	**Eprosartan** (Teveten)
Quinapril (Accupro, Accuretic)	**Azilsartan** (Edarbi)
Trandolapril (Gopten, Tarka)	
Imidapril hydrochloride (Tanatril)	
Moexipril hydrochloride (Perdix)	

Questions about the study treatments may be directed to the coordinating centre in Oxford

UK HARP-III, Clinical Trial Service Unit (CTSU)
Richard Doll Building, University of Oxford
Roosevelt Drive, OXFORD, OX3 7LF

Tel: 0800 585323 (Freefone) or +44 (0)1865 765615 (from outside UK)
Email: harp3@ctsu.ox.ac.uk Website: www.harp3trial.org

Study Treatment Information Leaflet

This leaflet contains important information relating to your UK HARP-III study treatment. Please read all the information contained in this leaflet very carefully. If you have any questions about your study treatment, please feel free to call a UK HARP-III study nurse or doctor on: **Freefone 0800 585323**

Please keep this information leaflet in a safe place for future reference.

Throughout the study you will be provided with two types of study treatment:

- Tablets of **LCZ696 200 mg** or **matching placebo** ("dummy")
- Capsules of **irbesartan 150 mg** or **matching placebo**

Initial Run-in phase

After your first study visit you will enter the "Run-in phase". You will be issued with a pack of **Type F Run-in Treatment** containing two bottles of study treatment, one labelled as bottle **A** and one as bottle **B**.

i. Bottle **A** will contain an 8 week supply of tablets of LCZ696 200 mg or placebo and will be identified by a blue label.
ii. Bottle **B** will contain an 8 week supply of capsules of irbesartan 150 mg or placebo and will be identified by a white label.

Long-term phase

At your second study visit, if you proceed into the long-term part of the study you will be randomly allocated to receive tablets containing either LCZ696 200 mg or matching placebo tablets. If you are allocated active tablets of LCZ696 you will also receive placebo irbesartan capsules, and if you are allocated to receive placebo tablets of LCZ696 you will then receive active irbesartan capsules.

At your second visit, and your 3, 6 and 9 month visits, you will be issued with a pack of **Type R Randomised Treatment** with two bottles of study treatment containing your random allocation, one labelled as bottle **X** and one as bottle **Y**.

i. Bottle **X** will contain a 3 month supply of tablets of LCZ696 200 mg or placebo and will again be identified by a blue label.
ii. Bottle **Y** will contain a 3 month supply of capsules of irbesartan 150 mg or placebo and will again be identified by a white label.

About your study tablets and capsules

LCZ696 200 mg or matching placebo tablets
and
Irbesartan 150 mg or matching placebo capsules

- Oral use.
- Do not store above 25°C.
- Protect from moisture.
- Keep out of the sight and reach of children.
- For clinical trial use only.

- During the Run-in phase, following your first visit, you should take **1 tablet daily** from bottle **A** (LCZ696 or placebo) and **1 capsule daily** from bottle **B** (irbesartan or placebo).
- For the first 2 weeks after your second visit you should take **1 tablet daily** from bottle **X** (LCZ696 or placebo) and **1 capsule daily** from bottle **Y** (irbesartan or placebo).
- Your study nurse will have arranged for you to have a blood test done towards the end of this 2 week period. This is important so please contact your study nurse or the UK HARP-III office if you cannot make this appointment.
- After 2 weeks, you should start taking **1 tablet twice daily** from bottle **X** (LCZ696 or placebo) and **2 capsules once daily** from bottle **Y** (irbesartan or placebo), unless advised otherwise.

  Suggested treatment schedule:

	Morning	**Evening**
Bottle X (LCZ696 or placebo)	1 tablet	1 tablet
Bottle Y (Irbesartan or placebo)	2 capsules	Nil

- If you forget to take either type of tablet or capsule at your usual time, you may still take it later the same day. However, if you miss a whole day or

more, do not make up for the missed tablets or capsules. Instead, leave them in the bottle and continue from the day you restart.

- If you think you will reach the end of your bottles before your next clinic visit or if you lose your study treatment, please contact your study nurse or call the UK HARP-III office and we will arrange for replacement treatment to be provided.
- Please return unused tablets and capsules at each clinic visit.
- For women: if you become pregnant while taking the study tablets please stop them immediately and inform your local study nurse or call **Freefone 0800 585323** as soon as possible.

Possible side effects

Both LCZ696 and irbesartan may cause raised potassium levels in your blood. This will not cause symptoms so it is important that you attend your UK HARP-III study visits so that this can be checked. Please inform your study nurse or the UK HARP-III office if you cannot attend your study visit.

Both LCZ696 and irbesartan may lower your blood pressure. If you feel light-headed or dizzy on standing, please inform your study nurse or the UK HARP-III office. We can arrange for you to see your study nurse in the next few days for a review if we think it is necessary.

Further information about side effects of LCZ696 is given in the **UK HARP-III Participant Information Leaflet**. If any new information arises during the course of the study staff will discuss this with you. Updated information will also be available on the study website (**www.harp3trial.org**).

If you develop any symptoms that you think are related to your study medication, please contact your **study nurse** or call **Freefone 0800 585323** for further advice. Should any side effect of the study treatment become intolerable for you, you would, of course, be free to stop either type of study tablet at any time.

If at any time you develop vomiting or diarrhoea, please temporarily stop all study treatment until the symptoms have settled.

Appendix 6:

UK HARP-III trial recruitment questionnaire.

UK HARP-III Recruitment Survey

Site Name or number: ☐

Dear UK HARP-III Nurses,

Many thanks for all your hard work and efforts with recruiting patients for UK HARP-III and getting us across the finish line. As you know, in UK HARP-III we were also piloting new methods to recruit patients into the trial.

We recognise that each site has different ways of delivering clinical research and as such not all sites had the resources/databases to follow our proposed method of recruitment. However, we would be very grateful if you could complete this short feedback form on the methods you used to recruit patients for UK HARP-III, as this would help us plan future large-scale trials in nephrology.

Many thanks and best wishes,

The UK HARP-III Team

Identification of eligible participants

What was the *principal* method you used to identify potentially patients? (tick one)

- Consultant referral ☐
- Manually searching clinic lists ☐
- Using an IT search of your unit's renal database (if you have one) ☐
- Using a search of some other database/spreadsheet of patients attending clinics ☐
- Patients self-referring (e.g. from seeing a poster in out-patients) ☐
- Other, please specify ☐

_____

Were there any other methods that you use to identify potentially patients? (tick as many as appropriate)

- Direct consultant referral ☐
- Manually searching clinic lists ☐
- Using an IT search of your unit's renal database (if you have one) ☐
- Using a search of some other database/spreadsheet of patients attending clinics ☐
- Patients self-referring (e.g. from seeing a poster in out-patients) ☐
- Other, please specify ☐

_____

For all those patients who were potentially eligible, who did the pre-screening to help exclude those patients likely not to be eligible?

- Local study doctor/patients usual nephrologist ☐
- Study nurse ☐
- Both ☐

Invitation

What was the main method by which potentially eligible patients received their UK HARP-III invitation and patient information sheet?

- Provided in clinic by LLI/Study nurse or usual nephrologist ☐
- Posted to participant using mail-merge tools provided ☐
- Posted without using mail-merge ☐
- Other, please specify ☐

_____

If you used the mail-merging tools provided, did you do this yourself or did you have administrative help to do this?

- Did it yourself ☐
- Administrative and/or clerical support ☐
- Both ☐

Follow-up calls

Were you able to contact patients after they received their invitation to participate?

- Yes ☐
- No ☐

Who made the follow-up phone calls? (tick as many as appropriate)

- Study nurse ☐
- Admin support ☐
- Local study doctor ☐

How easy did you find making the calls and speaking to potentially eligible patients?

- Very easy ☐
- Easy ☐
- Neither easy nor difficult ☐
- Difficult ☐
- Very difficult ☐

General information on your renal unit

Does your renal unit have an electronic database of *all* CKD renal patients (not *just* those on dialysis/transplant)?

- Yes ☐
- No ☐

Approximately how many patients does your renal unit care for:

With CKD (not on RRT)	
With a transplant	
On dialysis (HD and PD)	
In total	

What population size/geographical area does your renal unit roughly cover?

_____

Appendix 7:

UK HARP-III trial participant reminder card.

6 and 9 month visits

On the morning of each of these appointments please collect a sample of the urine you pass first thing, and bring it to your appointment in the small specimen pot provided.

The appointment for your **6 month** visit to the study clinic is:

_____

The appointment for your **9 month** visit to the study clinic is:

_____

After these study clinic visits if your blood tests are satisfactory and you are not experiencing any problems you will continue to take **one tablet** from **Bottle X twice** a day and **two capsules** from **Bottle Y once** a day.

Preparation for Final Visit

Shortly before your final study visit the study nurse will arrange for you to have another test to measure your kidney function (measured GFR).

This appointment is on

Date: _____Time: _____

Location of test:

"Remember to contact the study nurse if any of your regular medications are changed or new tablets are started."

12 month (final) visit

The appointment for your **final** visit to the study clinic is:

_____

On the morning of your appointment please collect a sample of the urine you pass first thing, and bring it to your appointment in the small specimen pot provided. Also, please bring back all your study treatment bottles.

If necessary, please make an appointment with your GP prior to this visit to ensure you have a supply of any tablets you stopped taking when you entered the study. Your nurse will advise you on this and when to restart them. NB please do not restart them before your final visit.

What to take to every study visit

- All bottles of study tablets
- A list of all your regular medications
- Sample of urine you pass first thing in the morning
- Details of any illnesses or hospital admissions since your last study visit

If you need to change an appointment please contact your study nurse:

Phone number:

Email:

UK HARP-III
UK Heart and Renal Protection

Participant Reminder Card

Participant name:

Participant ID:

UK HARP-III Coordinating Centre (CTSU)
Richard Doll Building
University of Oxford
Old Road Campus
Oxford, OX3 7LF

24-hour Freefone: 0800 585323
e-mail: harp3@ctsu.ox.ac.uk

After Screening (1st Visit)

Your study nurse will have explained to you that we would like you to stop taking the following blood pressure medication during the study:

_____

Instead you need to take **one tablet** from Bottle A and **one capsule** from Bottle B once daily.

If you have agreed to provide a **24-hour urine sample** this needs to be collected **2 days** before your next appointment, so start on:

_____

How to collect this sample

It does not matter how much or little urine is passed each time, as long as it is all collected.

Completely empty your bladder first thing in the morning and discard that urine, but note the time e.g. 06:15.

For the next 24 hours (during the day and night) collect all the urine you pass in the container provided.

At the end of the 24 hours (e.g. the next morning at 06:15) you empty your bladder collecting that urine too. The collection is now complete. You need to take it to your 2nd appointment (randomization).

Measured GFR test

Shortly before your randomization visit the study nurse will arrange for you to have a test to measure your kidney function. This appointment is:

Date: _____time:_____

Location:

The appointment for your randomization visit at the study clinic is:

_____

On the morning of your appointment please collect a sample of the urine you pass first thing, and bring it to your appointment in the small specimen pot provided.

After this visit, initially you should take **one tablet** from the **Bottle X and one capsule** from the **Bottle Y** that have been provided today.

Two weeks after this visit you need to have a blood test either at the renal clinic or your GP, to check your kidney function. **Date for blood test**:

_____

If the test results are satisfactory you will need to start taking the full dose of the study tablets: **one tablet** from **Bottle X twice a day** and **two capsules** from **Bottle Y once a day**.

1 month visit

The appointment for **1 month** visit to the study clinic is:

_____

On the morning of your appointment please collect a sample of the urine you pass first thing, and bring it to your appointment in the small specimen pot provided.

After this study clinic visit if your blood tests are satisfactory and you are not experiencing any problems you will continue to take **one tablet** from **Bottle X twice** a day and **two capsules** from **Bottle Y once** a day.

3 month visit

The appointment for your **3 month** to the study clinic is:

_____

On the morning of your appointment collect a sample of the urine you pass first thing, and bring it to your appointment in the small specimen pot provided.

Please do not take your study tablets on the morning of this appointment.

Instead take the tablets to the clinic with you. The study nurse will be taking a blood test, after which you will be able to take your tablets.

If your blood tests are satisfactory and you are not experiencing any problems the study nurse will issue you with a new supply of tablets and collect the first set of bottles. You will continue to take **one tablet** from **Bottle X twice a day** and **two capsules** from **Bottle Y once a day**.

Appendix 8:

UK HARP-III trial study participation card.

UK HARP-III Patient Participation Card (Randomization)

UK HARP-III
UK Heart and Renal Protection III

UNIVERSITY OF OXFORD

Name:

Centre number:

Participant number:

UK HARP-III PPC V3.0_2015-05-11

UK HARP-III
UK Heart and Renal Protection III

UNIVERSITY OF OXFORD

This patient is participating in UK HARP-III. This involves taking either LCZ696 200mg (an angiotensin receptor-neprilysin inhibitor) twice daily and placebo irbesartan once daily, or placebo LCZ696 twice daily and active irbesartan 150-300mg, once daily. Participation in the study will be for about 13 months. The study started in 2014.

Enquiries to: UK HARP-III Coordinating Centre (CTSU)
Richard Doll Building, University of Oxford,
Old Road Campus, Oxford, OX3 7LF
24-hour Freefone: 0800 585323

Appendix 9:

UK HARP-III trial approvals (Ethics, MHRA, ARSAC).

Health Research Authority
NRES Committee East Midlands - Nottingham 2
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Telephone:
Facsimile:

05 December 2013

Dr Richard Haynes
Clinical Research Fellow
Clinical Trial Service Unit, Oxford University
CTSU, Richard Doll Building, Old Road Campus
Roosevelt Drive, Headington
Oxford
OX3 7LF

Dear Dr Haynes,

Study title:	**Randomized multicentre pilot study of LCZ696 versus irbesartan in patients with chronic kidney disease: UK Heart and Renal Protection (HARP)-III**
REC reference:	**13/EM/0434**
Protocol number:	**CTSUHARP3**
EudraCT number:	**2013-004205-89**
IRAS project ID:	**135727**

The Research Ethics Committee reviewed the above application at the meeting held on 25 November 2013. Thank you for attending to discuss the application.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Manager- Liza Selway on 0115 8839695

Ethical opinion

The chair introduced himself and the committee and thanked the researchers for attending the meeting

The committee asked the researchers if participants call for advice who is the named contact person for participants to contact as the Patient Information Sheet, does not provide a named contact. The researchers advised that the generic contact number links to a core of advisors who will have a basic knowledge of the trial and the procedures. They will be in a position to provide support to participants if required. The Chief Investigator will be available to support if

necessary

The committee advised the researchers that the process for the wash out needs to be made clearer to participants within the Participant Information Sheet. The researchers advised that participants will be requested to stop any current ACE inhibitors, angiotensin receptor blockers or direct renin inhibitor therapy. Once the participant has gone through the wash out period then placebo drugs are given to try out for a period of four to seven weeks. The committee queried if participants would be at risk during this process and the researchers advised that only the blood pressure may be of concern. Consequently regular blood pressure measurements will be taken and an alternative blood pressure drug could be given

The committee asked the researcher if other organisations would be reviewing the participants data as reflected in section A36 in the IRAS application form. The researchers advised that data will be shared but participants data will not be identified

The committee discussed the need for the Consent Form to request permission to inform the GP. The researchers advised that due to the nature of the trial then the GP would be informed and the section on the Consent Form enables the research team to confirm that they have discussed with the participant that the GP will be kept up to date

The committee asked the researcher who will be sending the invitation letter out to potential participants. The researchers advised that the letter will be send from either the participants GP or from their local health trust and that the local investigator will be a signatory on the letter

The committee discussed with the researcher the option of using a reply slip. The researchers acknowledged that this was a good idea but advised the committee that they did not have a postage budget so are unable to offer this service

The committee praised the researchers for the Participant Information Sheet which was felt to be well written in lay terms and very readable.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

Non NHS sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

The Participant Information Sheet

1. Insert PALS contact details to include the Invitation letter
2. Page 2, "what will happen to me if I take part" 3rd paragraph, change the sentence to read, "This will allow you and the UK HARPIII doctors and nurses to be sure the routine of taking these particular tablets suits you"
3. Study Timetable – Early Phase, insert – "During this period you have the chance to try the study treatments out and ensure the routine of taking these tablets suits you".

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett ([redacted]), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Advertisement	1.0	28 October 2013
Covering Letter		05 November 2013
Evidence of insurance or indemnity		01 August 2013
GP/Consultant Information Sheets		28 October 2013
Investigator CV		02 October 2013
Investigator's Brochure	11	13 March 2012
Letter from Sponsor		05 November 2013
Letter of invitation to participant	1.0	28 October 2013
Other: Summary of Product Characteristics - Irbesartan (Approvel)		14 August 2013
Participant Consent Form	1.0	28 October 2013
Participant Information Sheet	1.0	28 October 2013
Protocol	1.0	28 October 2013
REC application	135727/521797/1/326	04 November 2013
Sample Diary/Patient Card	1.0	28 October 2013

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/EM/0434	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

[redacted]

Dr Martin Hewitt
Chair

Email: NRESCommittee.EastMidlands-Nottingham2@nhs.net

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*
"After ethical review – guidance for researchers" SL-AR1

Copy to: *Ms Heather House*

NRES Committee East Midlands - Nottingham 2

Attendance at Committee meeting on 25 November 2013

Committee Members:

Name	*Profession*	*Present*	*Notes*
Ms Gill Bumphrey	Clinical Trials Pharmacist	Yes	
Miss Shamim Byrne	Gynaecologist/Obstetrician	Yes	
Dr Frances Game	Consultant Physician	No	
Dr Martin Hewitt (Chair)	Consultant Paediatric Oncologist	Yes	
Dr Asam Latif	Research Pharmacist	No	
Mrs Veronica Lyon	Lay member	Yes	
Dr Simon Roe	Consultant Nephrologist	Yes	
Dr John Shaw	Lay Member	Yes	
Miss Catherine Shenton	Lay Member	Yes	
Mrs Sally Ann Smith	Retired Audit Manager	Yes	
Ms Margret Vince	Translator	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Miss Liza Selway	REC Manager

Health Research Authority
NRES Committee East Midlands - Nottingham 2
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0115 8839697

17 December 2013

Dr Richard Haynes
Clinical Research Fellow
Clinical Trial Service Unit, Oxford University
CTSU, Richard Doll Building, Old Road Campus
Roosevelt Drive, Headington
Oxford
OX3 7LF

Dear Dr Haynes,

Study title:	**Randomized multicentre pilot study of LCZ696 versus irbesartan in patients with chronic kidney disease: UK Heart and Renal Protection (HARP)-III**
REC reference:	**13/EM/0434**
Protocol number:	**CTSUHARP3**
EudraCT number:	**2013-004205-89**
IRAS project ID:	**135727**

Thank you for your letter of 16th December 2013. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 05 December 2013.

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Participant Information Sheet	1.1 (Clean and tracked)	09 December 2013

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
Advertisement	1.0	28 October 2013
Covering Letter		05 November 2013
Evidence of insurance or indemnity		01 August 2013

GP/Consultant Information Sheets		28 October 2013
Investigator CV		02 October 2013
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Letter from Sponsor		05 November 2013
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Participant Information Sheet	1.1	09 December 2013
Protocol	1.0	28 October 2013
REC application	135727/521797/1/326	04 November 2013
Sample Diary/Patient Card	1.0	28 October 2013

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

13/EM/0434 **Please quote this number on all correspondence**

Yours sincerely,

Rachel Nelson
REC Assistant

E-mail: NRESCommittee.EastMidlands-Nottingham2@nhs.net

Copy to: Ms Heather House

MHRA
Regulating Medicines and Medical Devices

MHRA
151 Buckingham Palace Road
London SW1W 9SZ
United Kingdom

mhra.gov.uk

Mr R Haynes
UNIVERSITY OF OXFORD
Richard Doll Building
Old Road Campus
Oxford
OX3 7LF
UNITED KINGDOM

15/09/2014

Dear Mr R Haynes

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference:	21439/0243/001-0001
Eudract Number:	2013-004205-89
Product:	LCZ696
Protocol number:	CTSUHARP3

NOTICE OF ACCEPTANCE OF AMENDED REQUEST

I am writing to inform you that the Licensing Authority accepts your amended request for a clinical trial authorisation (CTA), received on 08/09/2014.

The authorisation is effective from the date of this letter although your trial may be suspended or terminated at any time by the Licensing Authority in accordance with regulation 31. You must notify the Licensing Authority within 90 days of the trial ending.

Finally, you are reminded that a favourable opinion from the Ethics Committee is also required before this trial can proceed; changes made as part of your amended request may need to be notified to the Ethics Committee.

Yours sincerely,

Clinical Trials Unit
MHRA

From: ARSAC
Sent: 03 January 2014 08:23
To: ctrg@admin.ox.ac.uk
Cc: EAST MIDLANDS - NOTTINGHAM 2 (NRESCommittee.eastmidlands-nottingham2@nhs.net)
Subject: ARSAC PRA approval - IRAS ID 135727, REC Ref 13/EM/0434

I am writing regarding your Preliminary Research Assessment (PRA) form date **04 11 13** entitled:

Randomized multicentre pilot study of LCZ696 versus irbesartan in patients with chronic kidney disease: UK Heart and Renal Protection (HARP)-III

This has been approved by the ARSAC and certificate(s) for this study will be issued following receipt of complete applications from the site(s) involved.

If you should have any queries, then please do not hesitate to contact me at this email address, quoting the IRAS ID on all correspondence.

Yours sincerely

Elaine Gilder

ARSAC Support Unit
Centre for Radiation, Chemical and Environmental Hazards
Public Health England
Chilton, Didcot, Oxfordshire OX11 0RQ
arsac@phe.gov.uk
Tel: 01235 825006/7 (administration); 01235 825004/1 (scientific)
www.arsac.org.uk

Appendix 10:

UK HARP-III trial main results publication and Supplementary data:

Haynes R, Judge PK, Staplin N, Herrington WG, Storey BC, Bethel A, Bowman L, Brunskill N, Cockwell P, Hill M, Kalra PA, McMurray JJV, Taal M, Wheeler DC, Landray MJ, Baigent C. Effects of Sacubitril/Valsartan Versus Irbesartan in Patients With Chronic Kidney Disease. *Circulation* 2018;138(15):1505-1514.

Circulation

ORIGINAL RESEARCH ARTICLE

Effects of Sacubitril/Valsartan Versus Irbesartan in Patients With Chronic Kidney Disease

A Randomized Double-Blind Trial

Editorial, see p 1515

Richard Haynes, DM
Parminder K. Judge, MRCP
Natalie Staplin, PhD
William G. Herrington, MD
Benjamin C. Storey, MRCP
Angelyn Bethel, MD
Louise Bowman, MD
Nigel Brunskill, PhD
Paul Cockwell, PhD
Michael Hill, PhD
Philip A. Kalra, MD
John J.V. McMurray, MD
Maarten Taal, MD
David C. Wheeler, MD
Martin J. Landray, PhD
Colin Baigent, FRCP
On behalf of the UK HARP-III Collaborative Group

BACKGROUND: Sacubitril/valsartan reduces the risk of cardiovascular mortality among patients with heart failure with reduced ejection fraction, but its effects on kidney function and cardiac biomarkers in people with moderate to severe chronic kidney disease are unknown.

METHODS: The UK HARP-III trial (United Kingdom Heart and Renal Protection-III), a randomized double-blind trial, included 414 participants with an estimated glomerular filtration rate (GFR) 20 to 60 mL/min/1.73 m^2 who were randomly assigned to sacubitril/valsartan 97/103 mg twice daily versus irbesartan 300 mg once daily. The primary outcome was measured GFR at 12 months using ANCOVA with adjustment for each individual's baseline measured GFR. All analyses were by intention to treat.

RESULTS: In total, 207 participants were assigned to sacubitril/valsartan and 207 to irbesartan. Baseline measured GFR was 34.0 (SE, 0.8) and 34.7 (SE, 0.8) mL/min/1.73 m^2, respectively. At 12 months, there was no difference in measured GFR: 29.8 (SE 0.5) among those assigned sacubitril/valsartan versus 29.9 (SE, 0.5) mL/min/1.73 m^2 among those assigned irbesartan; difference, −0.1 (0.7) mL/min/1.73 m^2. Effects were similar in all prespecified subgroups. There was also no significant difference in estimated GFR at 3, 6, 9, or 12 months and no clear difference in urinary albumin:creatinine ratio between treatment arms (study average difference, −9%; 95% CI, −18 to 1). However, compared with irbesartan, allocation to sacubitril/valsartan reduced study average systolic and diastolic blood pressure by 5.4 (95% CI, 3.4–7.4) and 2.1 (95% CI, 1.0–3.3) mm Hg and levels of troponin I and N terminal of prohormone brain natriuretic peptide (tertiary end points) by 16% (95% CI, 8–23) and 18% (95% CI, 11–25), respectively. The incidence of serious adverse events (29.5% versus 28.5%; rate ratio, 1.07; 95% CI, 0.75–1.53), nonserious adverse reactions (36.7% versus 28.0%; rate ratio, 1.35; 95% CI, 0.96–1.90), and potassium ≥5.5 mmol/L (32% versus 24%, *P*=0.10) was not significantly different between randomized groups.

CONCLUSIONS: Over 12 months, sacubitril/valsartan has similar effects on kidney function and albuminuria to irbesartan, but it has the additional effect of lowering blood pressure and cardiac biomarkers in people with chronic kidney disease.

CLINICAL TRIAL REGISTRATION: URL: http://www.isrctn.com. Unique identifier: ISRCTN11958993.

Key Words: chronic kidney disease ■ neprilysin inhibition ■ renin-angiotensin system

Sources of Funding, see page 1513

https://www.ahajournals.org/journal/circ

Clinical Perspective

What Is New?

- The UK HARP-III trial (United Kingdom Heart and Renal Protection-III) has demonstrated that, in a wide range of people with proteinuric chronic kidney disease, adding neprilysin inhibition to angiotensin II receptor blockade has no additional effect on kidney function or albuminuria compared with irbesartan.
- The tolerability and safety profiles of the 2 treatments were not different. However, compared with irbesartan, sacubitril/valsartan further reduces both blood pressure and biomarkers of cardiovascular risk (troponin I and N-terminal pro-B-type natriuretic peptide).

What Are the Clinical Implications?

- UK HARP-III raises the hypothesis that sacubitril/valsartan could be an acceptable treatment to reduce cardiovascular risk in people with chronic kidney disease, a high-risk population with an unmet need.

Patients with chronic kidney disease (CKD) are at increased risk of both progression to end-stage renal disease and cardiovascular events compared with patients with normal kidney function.[1–3] Randomized controlled trials have shown that renin-angiotensin system (RAS) inhibitors slow the progression of diabetic and nondiabetic proteinuric CKD,[4–7] and lowering low-density lipoprotein cholesterol reduces the risk of atherosclerotic vascular events.[8] However, despite such treatments, a significant risk of progression to end-stage renal disease and cardiovascular events remains. In particular, patients with CKD are at increased risk of events related to structural heart disease (such as heart failure and arrhythmias), with many dying of cardiovascular disease before they reach end-stage renal disease.[9]

Natriuretic peptides have a range of potentially beneficial effects, including natriuresis, diuresis, vasodilatation, and inhibition of RAS.[10,11] Neprilysin (NEP or neutral endopeptidase) is the key enzyme responsible for degrading natriuretic peptides and other vasoactive peptides, such as angiotensin II, bradykinin, endothelin, and substance P.[10,12] Although inhibition of NEP (NEPi) raises concentrations of circulating natriuretic peptides, it also leads to reflex RAS activation and inhibits angiotensin II breakdown, counteracting any potentially beneficial effects, so NEPi must be combined with RAS inhibition. Combinations of NEPi and angiotensin-converting enzyme inhibitors are associated with a high risk of angioedema (because of excessive inhibition of bradykinin degradation),[13] so the chosen method of RAS inhibition for use with NEPi is an angiotensin receptor blocker. Sacubitril/valsartan, which combines an angiotensin receptor blocker (valsartan) with a NEPi (sacubitril), was the first angiotensin receptor–neprilysin inhibitor to be developed.

The PARADIGM-HF trial (Prospective Comparison of an Angiotensin Receptor–Neprilysin Inhibitor With Angiotensin-Converting Enzyme Inhibitors to Determine Impact on Global Mortality and Morbidity in Heart Failure) showed that sacubitril/valsartan reduced the risk of cardiovascular mortality among patients with heart failure with reduced ejection fraction when compared with the angiotensin-converting enzyme inhibitor enalapril (hazard ratio, 0.80; 95% CI, 0.71–0.89).[14] Several trials in populations with heart failure, including PARADIGM-HF, suggest that sacubitril/valsartan slows the decline in kidney function compared with RAS inhibition alone, but that it slightly increased albuminuria.[15–17] Animal studies have shown that combining NEP and RAS inhibition can reduce proteinuria and histological evidence of kidney damage.[18–21] The UK HARP-III trial (United Kingdom Heart and Renal Protection-III) aimed to compare the effects of sacubitril/valsartan versus irbesartan (a licensed angiotensin receptor blocker for diabetic nephropathy) on kidney function and other outcomes in people with CKD.

METHODS

Trial Design and Participants

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results from the Richard Doll Centenary Archive according to the Nuffield Department for Population Health's Data Sharing Policy.[22] Details of the UK HARP-III trial objectives, design, and methods have been reported previously.[23] Ethical (Nottingham Research Ethics Committee 2 [13/EM/0434]) and regulatory approvals were obtained before the enrollment of any study participants. Participants ≥18 years of age were eligible to participate if they had CKD with either (1) an estimated glomerular filtration rate (eGFR) of ≥45 and <60 mL/min/1.73 m^2 and a urine albumin:creatinine ratio (uACR) >20 mg/mmol (177 mg/g), or (2) an eGFR of ≥20 and <45 mL/min/1.73 m^2 (regardless of uACR).

Potentially eligible participants attended a screening visit at which medical history and eligibility criteria were checked, written informed consent was obtained, and blood and urine samples were taken for local laboratory analysis. Any current RAS inhibitor was stopped, and the participant entered the 4- to 7-week single-blind prerandomization run-in phase, during which they took 1 placebo sacubitril/valsartan tablet and 1 placebo irbesartan capsule daily. The aims of the run-in phase were to (1) enable a washout of any angiotensin-converting enzyme inhibitors before introduction of NEPi (to reduce the risk of angioedema), (2) allow a comparison of the acute effects of the study treatments on eGFR, and (3) identify and exclude those less likely to adhere to study treatment and

trial procedures before randomization to maintain statistical sensitivity.[24,25]

Randomization and Masking

At the end of the run-in period, glomerular filtration rate (GFR) was measured, and willing and eligible participants were randomized 1:1 to sacubitril/valsartan or irbesartan by an internet-based system with minimized randomization (which helped ensure balance for categories of age, sex, systolic blood pressure, previous diabetes mellitus, eGFR, and uACR).[23] Treatment allocation was concealed, so investigators, clinicians, and patients had no foreknowledge of the upcoming treatment allocation.[26] A double-dummy approach was used to ensure participants and study staff remained blind to treatment allocation: participants were issued 2 bottles of study treatments, 1 containing sacubitril/valsartan 97/103 mg or placebo tablets and the other containing irbesartan 150 mg or placebo capsules.[27]

Procedures

After randomization, participants were initially instructed to take 1 tablet and 1 capsule daily of study treatment (ie, either sacubitril/valsartan 97/103 mg or irbesartan 150 mg); this dosage was increased to sacubitril/valsartan 97/103 mg twice daily or irbesartan 300 mg once daily after 2 weeks unless potassium or change in kidney function precluded a dose increase. Study visits were scheduled at 1, 3, 6, 9, and 12 months after randomization (and additional visits arranged where necessary to monitor participant safety). At each follow-up, study staff sought information on all serious adverse events and any nonserious adverse events considered with reasonable probability to be related to study treatment. Compliance with study treatments was assessed by self-report, and blood pressure and weight were measured at every visit. Blood and urine samples were collected at every study visit for local analysis of creatinine, potassium, liver function tests (bilirubin, liver transaminase, and alkaline phosphatase), and uACR. Central laboratory assays of creatinine, uACR, and cardiac biomarkers (troponin I and NT-proBNP [N-terminal pro-B-type natriuretic peptide]) were conducted at randomization, 6 months, and 12 months. Additionally, participants were advised not to take their morning dose of study treatment on the day of their 3-month visit so that creatinine, uACR, and trough blood levels of sacubitril, sacubitrilat (the primary metabolite of sacubitril), and valsartan could be collected. GFR was measured at or just before the 12-month visit, and paper results of all GFR measurements were sent to the coordinating center for verification blind to treatment allocation. If participants were unwilling or no longer able to attend follow-up visits, information was obtained by telephone or from relatives or caregivers wherever possible. The original protocol specified that 360 participants would be followed for 6 months; before the completion of recruitment (and blind to any interim results), the steering committee decided to extend follow-up to 12 months (because of results from other trials suggesting that the effect on kidney function may take ≥9 months to fully emerge) and to increase the sample size to ≥400 participants (to increase the statistical power).

Laboratory Methods

GFR was measured in the study centers using ^{51}Cr-EDTA, ^{99m}Tc-DTPA (diethylenetriaminepentaacetic acid), or iohexol methods depending on local practice (with each center using the same method at baseline and 12 months). Creatinine was assayed in the central laboratory on a Beckman Coulter AU680 analyzer using a kinetic alkaline picrate method and calibrated using material traceable to isotope dilution mass spectrometry (using the National Institute of Standards and Technology Standard Reference Material 967); troponin I was measured by immunoassay on an Architect system and NT-proBNP by immunoassay on an Elecsys system.

Statistical Analysis

The primary outcome was measured GFR (mGFR), and ANCOVA was used to compare mean mGFR at 12 months between patients allocated sacubitril/valsartan and irbesartan patients, with adjustment for each individual's baseline mGFR.[28] Assuming a between-person SD in mGFR of 15 mL/min/1.73 m^2 and a correlation between an individual's baseline and follow-up mGFR of 0.8, randomization of 400 participants would provide ≥80% power (at P=0.05) to detect a difference in mGFR at the final follow-up (adjusted for baseline values) of 3 mL/min/1.73 m^2, even if 15% of participants discontinued allocated study treatment.

All analyses were performed according to the intention-to-treat principle among all randomized participants.[29,30] Comparisons of continuous outcomes were performed using ANCOVA adjusted for each participant's baseline value, after appropriate transformation if required. Multiple imputation methods were used to account for missing data.[31] Time-to-event analyses used log-rank methods to calculate event rate ratios, 95% CIs, and associated 2-sided P values.[29,30] Pharmacokinetic analyses involved multiple linear regression of each sacubitril/valsartan metabolite against a number of prespecified baseline variables, adjusted for time since the last dose of sacubitril/valsartan. The primary pharmacokinetic analysis restricted the dataset to those participants assigned sacubitril/valsartan who had last taken the drug 10 to 16 hours before the sample being collected. Further details (including secondary and tertiary outcomes) are available in the prespecified data analysis plan.[23] Analyses were done using SAS version 9.3 (SAS Institute) and R version 3.3.3 (www.R-Project.org).

RESULTS

Between November 1, 2014, and January 31, 2016, 620 participants attended screening visits, and 566 (91%) entered the prerandomization run-in (Figure 1). In total, 414 participants were randomized: 207 to sacubitril/valsartan and 207 to irbesartan. The mean age was 62.8 years (SD, 13.7), 298 (72%) were male, and the mean blood pressure was 146/81 mm Hg (Table 1). Mean eGFR at baseline was 35.5 (10.9) mL/min/1.73 m^2, and the median uACR was 54 (interquartile range, 11–153) mg/mmol (Table 1).

By 12 months, similar proportions of participants in each arm had stopped study treatment (33 [16%]

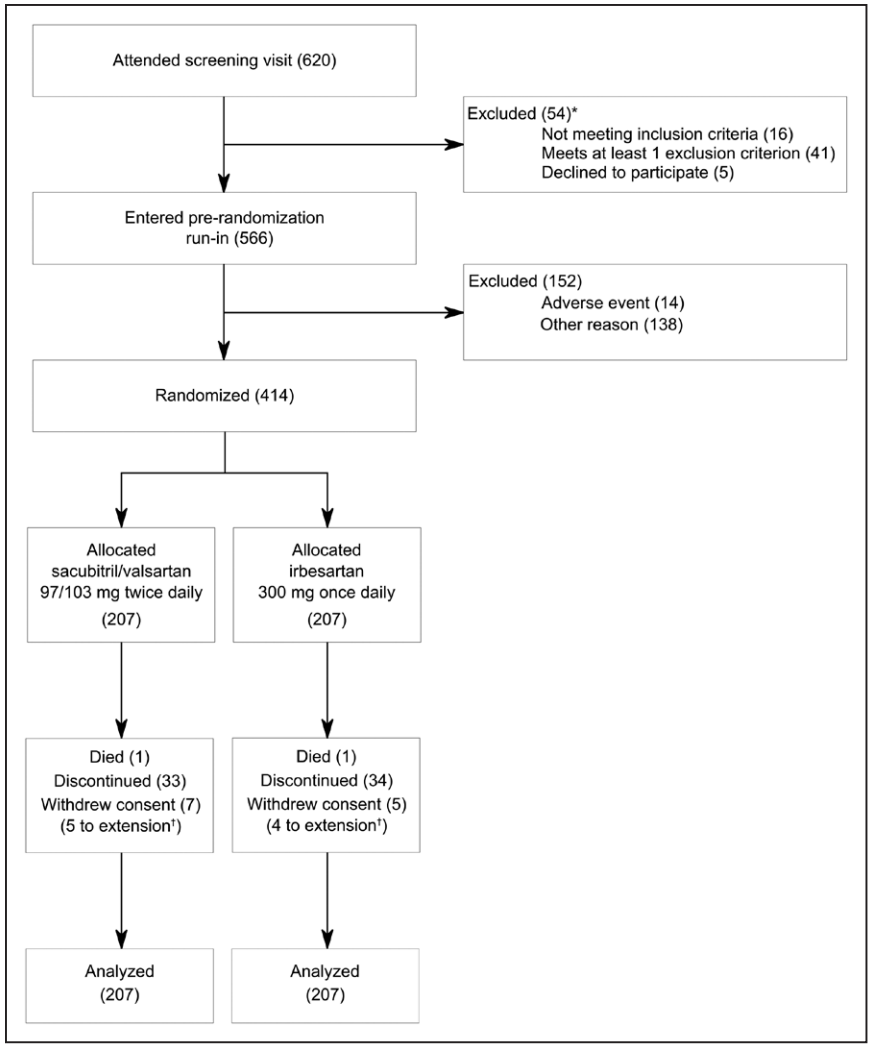

Figure 1. Flow of participants.
*Participants could report >1 reason. †Duration of the trial was increased from 6 to 12 months, and 9 participants did not consent to this extension and so completed follow-up at 6 months.

of those assigned sacubitril/valsartan and 34 [16%] of those assigned irbesartan), and the reasons for stopping full dose study treatment were similar. There was no excess of discontinuations because of serious adverse events, nonserious adverse reactions, or other reasons in those allocated sacubitril/valsartan (Table I in the online-only Data Supplement).

At 12 months, the mean (SE) mGFR was 29.8 (0.5) mL/min/1.73 m^2 among those assigned to the sacubitril/valsartan group compared with 29.9 (0.5) mL/min/1.7 $3m^2$ among those assigned irbesartan, a nonsignificant difference of 0.1 (0.7) mL/min/1.73 m^2 (P=0.86) (Table 2). Neither a prespecified complete case analysis (ie, without imputation: difference −0.4 [0.7] mL/min/1.73 m^2) nor an "on-treatment" analysis (difference −0.5 [0.7] mL/min/1.73 m^2) materially affected this finding. There was no evidence that the difference between sacubitril/valsartan and irbesartan in effect on mGFR differed by age (χ_1^2=0.45, P=0.50), sex (χ_1^2=0.70, P=0.4), baseline mGFR (χ_1^2=0.42, P=0.52), baseline uACR (χ_1^2=0.76, P=0.38), cause of kidney disease (χ_6^2=2.24, P=0.90), or any other prespecified baseline characteristic (Figure I in the online-only Data Supplement).

Compared with irbesartan, allocation to sacubitril/valsartan was not associated with any significant effect on eGFR at any time point (Figure 2). The rate of change in eGFR did not differ significantly between arms, whether measured from randomization to 12 months, from randomization to 3 months, or from 3 to 12 months (Table II in the online-only Data Supplement).

Allocation to sacubitril/valsartan produced a nonsignificant 9% (−18% to 1%, P=0.08) reduction in study-average uACR (Table 3) and was associated with a reduction in blood pressure compared with irbesartan. Overall, the mean systolic blood pressure was 5.4 (95% CI, −7.4 to −3.4) mm Hg lower, and the mean diastolic blood pressure was 2.1 (95% CI, −3.3 to −1.0) mm Hg lower among those allocated to sacubitril/valsartan (Table 3). Exploratory analyses did not show any differences in the intensity of nonstudy antihypertensive agents between the treatment arms during follow-up.

Table 1. Baseline Characteristics by Randomized Treatment Allocation

Variable	Sacubitril/ Valsartan (n=207)	Irbesartan (n=207)
Age at randomization, y		
Mean age (SD)	62.0 (14.1)	63.6 (13.4)
<50	37 (18%)	36 (17%)
≥50 to <70	97 (47%)	99 (48%)
≥70	73 (35%)	72 (35%)
Sex		
Male	148 (71%)	150 (72%)
Female	59 (29%)	57 (28%)
Ethnicity		
White	186 (90%)	191 (92%)
Black	3 (1%)	4 (2%)
South Asian	11 (5%)	7 (3%)
Other	7 (3%)	5 (2%)
Self-reported prior disease		
Coronary heart disease	21 (10%)	33 (16%)
Cerebrovascular disease	16 (8%)	15 (7%)
Peripheral vascular disease	22 (11%)	22 (11%)
Heart failure	8 (4%)	7 (3%)
Diabetes mellitus	81 (39%)	83 (40%)
Systolic blood pressure at randomization (mm Hg)		
Mean systolic blood pressure (SD)	146 (16)	146 (16)
<140	76 (37%)	85 (41%)
≥140 to <160	93 (45%)	84 (41%)
≥160	38 (18%)	38 (18%)
Diastolic blood pressure at randomization (mm Hg)		
Mean diastolic blood pressure (SD)	81 (11)	80 (11)
<80	96 (46%)	105 (51%)
≥80 to <90	68 (33%)	58 (28%)
≥90	43 (21%)	44 (21%)
Body mass index, kg/m^2		
Mean body mass index (SD)	30 (6)	31 (6)
<25	35 (17%)	33 (16%)
≥25 to <30	74 (36%)	73 (35%)
≥30	95 (46%)	100 (48%)
Not available	3	1
Medication		
Antiplatelet therapy	64 (31%)	75 (36%)
Oral anticoagulant	13 (6%)	15 (7%)
Diuretic	79 (38%)	85 (41%)
Calcium channel blocker	104 (50%)	103 (50%)
β-Blocker	50 (24%)	62 (30%)
α-Blocker	58 (28%)	55 (27%)
LDL-lowering agent	126 (61%)	137 (66%)
Use of RAS blockade at screening visit		
Yes	173 (84%)	166 (80%)
No	34 (16%)	41 (20%)
CKD-EPI estimated glomerular filtration rate at randomization, mL/min/1.73 m^2		
Mean (SD)	35.4 (11.0)	35.5 (11.0)
<30	79 (38%)	77 (37%)
≥30 to <45	86 (42%)	91 (44%)
≥45	41 (20%)	39 (19%)
Not available	1	0
Urine albumin:creatinine ratio at randomization, mg/mmol		
Geometric mean (≈SE)	34 (5)	34 (5)
Median (IQR)	52 (11–162)	56 (11–146)
<3	30 (14%)	28 (14%)
≥3 to <30	43 (21%)	45 (22%)
≥30	134 (65%)	134 (65%)
Cause of kidney disease		
Glomerular disease	60 (29%)	51 (25%)
Tubulointerstitial disease*	18 (9%)	32 (15%)
Diabetic kidney disease†	36 (17%)	47 (23%)
Hypertensive/renovascular disease†	18 (9%)	24 (12%)
Other systemic diseases affecting the kidneys†	1 (0%)	2 (1%)
Familial/hereditary nephropathies	30 (14%)	13 (6%)
Other known causes‡	5 (2%)	4 (2%)
Unknown‡	39 (19%)	34 (16%)
24-h urinary sodium excretion during run-in, mg/24 h		
Geometric mean (≈SE)	2245 (183)	2585 (187)
Median (IQR)	2484 (1794–3795)	2875 (1932–4232)
Not available	100	110

Values are n (%), mean (SD), geometric mean (≈SE), or median (IQR). CKD-EPI indicates Chronic Kidney Disease Epidemiology Collaboration; ERA-EDTA, European Renal Association-European Dialysis and Transplant Association; IQR, interquartile range; LDL, low-density lipoprotein; and RAS, renin–angiotensin system.

*Includes obstructive renal diseases.

†All considered systemic diseases affecting the kidney by the ERA-EDTA registry.

‡All considered miscellaneous renal disorders by the ERA-EDTA registry.

Allocation to sacubitril/valsartan was associated with significant reductions in levels of cardiac biomarkers compared with irbesartan. Study average NT-proBNP concentrations were 18% (−25 to −11%) lower and troponin I levels were 16% (−23% to −8%) lower among participants assigned sacubitril/valsartan (Table 3).

Using data from 87 participants who had taken their last dose of sacubitril/valsartan 10 to 16 hours previously, no significant determinants of sacubitril or valsartan concentration were identified (Table III in the online-only Data Supplement). However, kidney function was a major determinant of sacubitrilat

Table 2. Effect of Allocation to Sacubitril/Valsartan Versus Irbesartan on Measured Glomerular Filtration Rate at 12 Months

Follow-Up Visit	Mean mGFR (SE) (mL/min/1.73 m²)		Difference in Means (SE)*	*P* Value
	Sacubitril/ Valsartan (n=207)	Irbesartan (n=207)		
Randomization	34.0 (0.8)	34.7 (0.8)		
12 mo	29.8 (0.5)	29.9 (0.5)	–0.1 (0.7)	0.86

Where the difference between mGFR and central eGFR at the corresponding time point was more extreme than the first or 99th percentile of the distribution of differences, the value of mGFR was set to missing. Ten missing mGFR values at randomization had eGFR values at randomization imputed, and 41 missing mGFR values at 12 mo were imputed with the use of multiple imputation. For the 2 patients who commenced chronic dialysis during the study, a value of 0 was imputed for their 12-mo mGFR. eGFR indicates estimated glomerular filtration rate; and mGFR, measured glomerular filtration rate.

*Values are absolute differences in arithmetic means (SE). The 12-mo estimates and *P* values were derived from ANCOVA with adjustment for the randomization value.

concentration, with each 10 mL/min lower mGFR being associated with a 1485 (572–2397) ng/mL higher sacubitrilat concentration (Table III in the online-only Data Supplement).

Allocation to sacubitril/valsartan had no significant effect on fatal serious adverse events (1 [0.5%] versus 1 [0.5%]) or on any nonfatal serious adverse events (61 [29.5%] versus 59 [28.5%]; rate ratio, 1.07; 95% CI, 0.75–1.53; *P*=0.70) (Table IV in the online-only Data Supplement). One case of angioedema occurred in a participant allocated sacubitril/valsartan, but the participant did not attend hospital or require any specific treatment. There was no difference overall in the number of nonserious adverse reactions (76 [36.7%] versus 58 [28.0%]; rate ratio, 1.35; 95% CI, 0.96–1.90; *P*=0.08) (Table IV in the online-only Data Supplement). Allocation to sacubitril/valsartan was associated with higher rates of nonserious hypotension (17 [8.2%] versus 7 [3.4%]; rate ratio, 2.36; 95% CI, 1.06–5.26; *P*=0.04). There was no difference between treatments in the number of participants experiencing hyperkalemia (66 [32%] versus 50 [24%], *P*=0.10) or in the proportion experiencing a significant decline in eGFR (defined as ≥25% reduction; 71 [34%] versus 67 [32%], *P*=0.75) (Table 4). There were no cases of significant liver injury.

DISCUSSION

The UK HARP-III trial has shown that, compared with irbesartan, 12 months of treatment with sacubitril/valsartan did not significantly affect kidney function in people with CKD. Sacubitril/valsartan had no additional effect on albuminuria compared with irbesartan and was as well tolerated, with no major safety concerns identified. Sacubitril/valsartan was also found to reduce blood pressure and biomarkers of cardiovascular risk (troponin I and NT-proBNP) compared with irbesartan.

The kidney function results from the UK HARP-III trial do not confirm findings from the analyses of kidney disease progression outcomes from other NEPi trials among patients with heart failure. In a trial among patients with heart failure with preserved ejection fraction, kidney function declined more slowly with sacubitril/valsartan compared with valsartan.[15] In the large PARADIGM-HF trial, a marginally

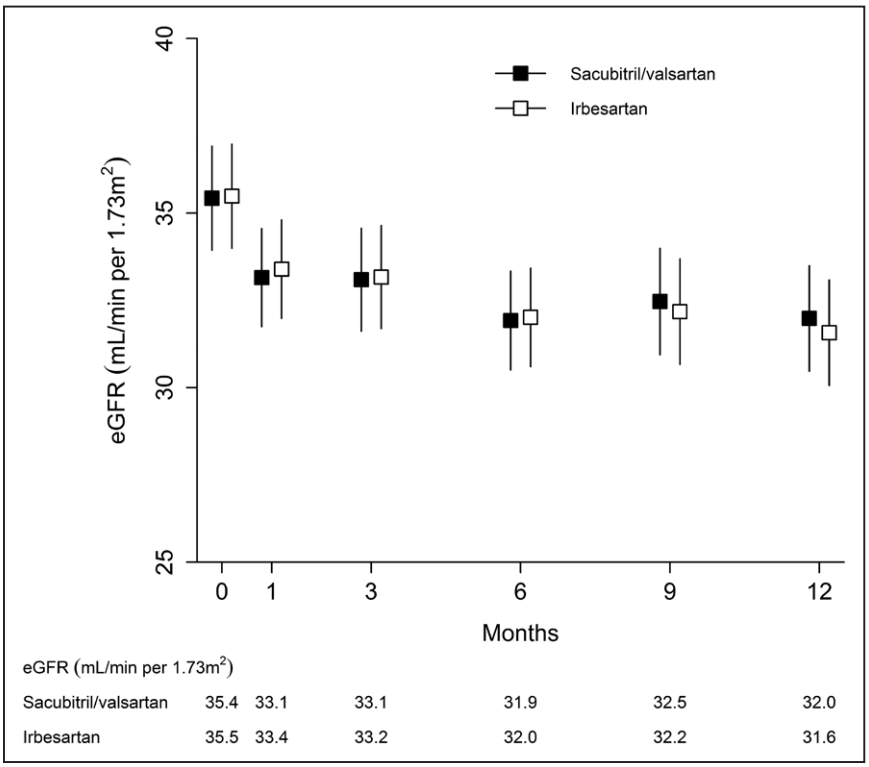

Figure 2. Effect of allocation to sacubitril/valsartan versus irbesartan on estimated glomerular filtration rate (eGFR). Creatinine measured in the central laboratory except for 1- and 9-month visits when creatinine was measured in the local laboratory. Error bars presented are 95% CIs.

Table 3. Effect of Allocation to Sacubitril/Valsartan Versus Irbesartan on Urinary Albumin:Creatinine Ratio, Systolic and Diastolic Blood Pressure, and Cardiac Biomarkers

Follow-Up Visit	Mean (SE)*		Difference in Means (95% CI)†	*P* Value
	Sacubitril/ Valsartan (n=207)	Irbesartan (n=207)		
Urinary albumin:creatinine ratio, mg/mmol				
Randomization	34.1 (4.6)	33.9 (4.5)		
3 mo	17.0 (1.0)	17.8 (1.0)	−4% (−19 to 12)	
6 mo	15.6 (1.0)	18.4 (1.1)	−15% (−28 to 0)	
12 mo	16.4 (1.2)	17.6 (1.3)	−6% (−23 to 14)	
Study average	16.3 (0.6)	17.9 (0.7)	−9% (−18 to 1)	0.08
Systolic blood pressure, mm Hg				
Randomization	146 (1.1)	146 (1.1)		
1 mo	129 (1.1)	132 (1.1)	−3.5 (−6.5 to −0.6)	
3 mo	129 (1.1)	137 (1.1)	−7.3 (−10.3 to −4.3)	
6 mo	128 (1.1)	135 (1.1)	−6.9 (−10.0 to −3.7)	
9 mo	130 (1.2)	134 (1.2)	−4.0 (−7.3 to −0.8)	
12 mo	128 (2.5)	133 (2.2)	−4.4 (−10.9 to 2.1)	
Study average	129 (0.8)	134 (0.7)	−5.4 (−7.4 to −3.4)	<0.001
Diastolic blood pressure, mm Hg				
Randomization	81 (0.8)	80 (0.8)		
1 mo	73 (0.6)	74 (0.6)	−0.8 (−2.5 to 0.9)	
3 mo	73 (0.6)	76 (0.6)	−2.6 (−4.3 to −0.9)	
6 mo	72 (0.6)	75 (0.6)	−2.5 (−4.2 to −0.8)	
9 mo	73 (0.6)	74 (0.6)	−1.8 (−3.6 to −0.1)	
12 mo	72 (1.6)	75 (1.3)	−2.2 (−6.2 to 1.9)	
Study average	73 (0.5)	75 (0.4)	−2.1 (−3.3 to −1.0)	<0.001
N-terminal pro-B-type natriuretic peptide, ng/L				
Randomization	254.5 (22)	250.9 (22)		
6 mo	175.6 (7.2)	219.7 (8.9)	−20% (−29 to −11)	
12 mo	210.2 (11)	247.5 (12)	−15% (−26 to −2)	
Study average	188.7 (6.0)	230.4 (7.3)	−18% (−25 to −11)	<0.001
Troponin I, ng/L				
Randomization	7.3 (0.5)	7.5 (0.5)		
6 mo	5.4 (0.2)	6.6 (0.2)	−19% (−27 to −10)	
12 mo	6.3 (0.4)	7.1 (0.4)	−11% (−24 to 4)	
Study average	5.7 (0.2)	6.8 (0.2)	−16% (−23 to −8)	<0.001

Any missing data were imputed with the use of multiple imputation.

*Geometric means (≈SE) are presented for urinary albumin:creatinine ratio and cardiac biomarkers, and arithmetic means (SE) are presented for blood pressure.

†Values are percentage changes in geometric means (95% CI) for urinary albumin:creatinine ratio and cardiac biomarkers, and absolute differences in arithmetic means (95% CI) for blood pressure. The estimates and *P* values at each follow-up visit were derived from ANCOVA with adjustment for the randomization value.

slower decline in eGFR was also observed with sacubitril/valsartan compared with enalapril (−1.3 [95% CI, −1.2 to −1.4] versus −1.8 [95% CI, −1.8 to −1.7] mL/min/1.73 m^2 per year; $P<0.0001$).[16] The lack of any additional effect of sacubitril/valsartan on kidney function in the UK HARP-III trial may reflect differing determinants of kidney disease progression in a proteinuric CKD population compared with heart failure populations. If cardiac function is a more important determinant of kidney function in a heart failure population than in proteinuric CKD, then a treatment that improves cardiac function, such as sacubitril/valsartan, might be more likely to affect kidney function in a heart failure population.

Studies using animal models of established kidney disease have found that combinations of NEP and

Table 4. Effect of Allocation to Sacubitril/Valsartan Versus Irbesartan on Biochemical Safety Data

Outcome	Sacubitril/ Valsartan (n=207)	Irbesartan (n=207)	*P* Value
Potassium, mmol/L			
≥5.5 to <6.0	44 (21%)	38 (18%)	
≥6.0 to <6.5	20 (10%)	7 (3%)	
≥6.5	2 (1%)	5 (2%)	
Total: Any potassium ≥5.5 mmol/L	66 (32%)	50 (24%)	0.10
Estimated glomerular filtration rate			
≥25% reduction in CKD-EPI eGFR*	71 (34%)	67 (32%)	0.75

Based on local laboratory measurements. CKD-EPI indicates Chronic kidney Disease Epidemiology Collaboration; and eGFR, estimated glomerular filtration rate.

*Compared to eGFR at randomization visit.

RAS inhibition are not associated with significant differences in GFR compared with isolated RAS inhibition.[18,19,21,32] However, histology results from these animals demonstrated that combined NEP/RAS inhibition was associated with greater reductions in histological markers of CKD progression (glomerulosclerosis and tubulointerstitial fibrosis), compared with isolated RAS inhibition.[12,18–20] It should be noted that the largest decline in eGFR was observed during the first month, likely attributable to the known glomerular hemodynamic effects of RAS inhibition. In the remaining 11 months of observation, eGFR decline was slow in both groups, implying that a longer observation period may have been necessary to observe the full effect on kidney function.

Allocation to sacubitril/valsartan did not increase albuminuria, in contrast with trials among patients with heart failure, among whom sacubitril/valsartan causes statistically significant (but clinically modest) increases in albuminuria (from a much lower baseline).[15] If similar increases in albuminuria had developed in people with proteinuric CKD, this would have been of concern because albuminuria is associated with an increased risk of progression to end-stage renal disease (although whether this association is directly causal remains uncertain).[33–35] Nonetheless, the lack of effect on albuminuria despite the observed blood pressure difference raises the possibility that the effect on systemic blood pressure does not lead to a reduction in intraglomerular pressure.

Sacubitril/valsartan lowered blood pressure compared with irbesartan. Similar additional reductions in blood pressure compared with RAS inhibition have been shown in populations with heart failure or hypertension.[14,36–39] These differences were observed in the context of a median of 1 other antihypertensive medication being used in addition to study treatment in both groups. It remains uncertain whether lowering blood pressure reduces the rate of progression of kidney disease,[40,41] but there is strong evidence that it reduces the risk of cardiovascular events.[41] Patients with CKD are at increased risk of cardiovascular events.[42] Indeed, most patients with CKD are at higher risk of cardiovascular mortality than progression to end-stage kidney disease (ie, dialysis or transplantation).[9] As kidney function declines, the nature of cardiovascular disease changes from a typical atherosclerotic phenotype to one of structural heart disease, which becomes increasingly prevalent such that 80% of patients starting dialysis have evidence of it.[43,44] The finding that NT-proBNP (an indicator of cardiac wall stress and not a substrate of neprilysin) and troponin levels (a marker of cardiomyocyte necrosis) were both lower among participants assigned sacubitril/valsartan compared with irbesartan has also been observed among patients with heart failure.[39,45,46] Recent animal data also demonstrated that sacubitril/valsartan attenuates cardiac hypertrophy and fibrosis in an animal model of CKD.[47] These findings raise the hypothesis that sacubitril/valsartan may have cardiovascular benefits among patients with advanced CKD and provides a rationale for a clinical outcome trial.

Sacubitril/valsartan was generally well tolerated, and no major hazards were observed; although there were numerically more nonserious adverse reactions in the sacubitril/valsartan group, this difference was not statistically significant. These randomized comparisons follow a placebo run-in during which 152/566 (26%) of participants withdrew, mostly for nonmedical reasons.[23] Compared with those allocated to irbesartan, participants allocated sacubitril/valsartan reported more symptoms of hypotension, which is expected given its larger blood pressure-lowering effect. Because kidney function is a major determinant of sacubitrilat concentration, it is possible that higher concentrations of sacubitrilat in this population contributed to this excess in hypotension. Both treatments had similar effects on the incidence of hyperkalemia, and no cases of significant liver injury were observed despite high blood concentrations of sacubitrilat resulting from reduced renal excretion. One participant allocated sacubitril/valsartan developed angioedema but did not require medical intervention, and it resolved spontaneously.

Study limitations include the short duration of follow-up and the sample size, which was not sufficiently large to test the effect of sacubitril/valsartan on clinical outcomes. The choice of comparator (irbesartan) also might have an effect on the interpretation of the results because it has a different pharmacological profile from valsartan and may provide more intense angiotensin receptor blockade.[48] This would suggest that the additional BP reduction and effects on cardiac biomarkers are an underestimate of the effect of neprilysin inhibition.

CONCLUSIONS

In conclusion, over 12 months in people with CKD, the combination of sacubitril and valsartan is well tolerated and has similar effects on kidney function and albuminuria to irbesartan, but it has additional blood pressure– and cardiac biomarker–lowering effects.

ARTICLE INFORMATION

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Disclosures

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Effects of sacubitril/valsartan versus irbesartan in patients with chronic kidney disease: United Kingdom Heart and Renal Protection (UK HARP)-III Trial

SUPPLEMENTAL MATERIAL

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Supplementary Table 1: Reasons for completely stopping randomized treatment, overall and by different types of adverse events

Reason for stopping	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	p-value
Serious adverse event			
Angiooedema	1 (0%)	0 (0%)	
Hyperkalaemia	0 (0%)	1 (0%)	
Acute kidney injury	1 (0%)	1 (0%)	
Gastrointestinal disorders	1 (0%)	0 (0%)	
Infections and infestations	1 (0%)	0 (0%)	
Pregnancy, puerperium and perinatal conditions	0 (0%)	1 (0%)	
Renal and urinary disorders	0 (0%)	1 (0%)	
Respiratory, thoracic and mediastinal disorders	0 (0%)	1 (0%)	
Surgical and medical procedures	0 (0%)	2 (1%)	
Subtotal: Any serious adverse event	4 (2%)	7 (3%)	0.54
Non-serious adverse reaction			
Hypotensive disorder	2 (1%)	2 (1%)	
Hyperkalaemia	4 (2%)	0 (0%)	
Acute kidney injury	1 (0%)	3 (1%)	
Abnormal liver function test	0 (0%)	0 (0%)	
Cardiac disorders	0 (0%)	1 (0%)	
Gastrointestinal disorders	3 (1%)	1 (0%)	
General disorders and administration site conditions	1 (0%)	2 (1%)	
Metabolism and nutrition disorders	1 (0%)	0 (0%)	
Musculoskeletal and connective tissue disorders	1 (0%)	0 (0%)	
Nervous system disorder	3 (1%)	2 (1%)	
Respiratory, thoracic and mediastinal disorders	0 (0%)	1 (0%)	
Skin and subcutaneous tissue disorders	2 (1%)	0 (0%)	
Subtotal: Any non-serious adverse reaction	18 (9%)	12 (6%)	0.34
Other reason			
Unable to attend clinic	2 (1%)	1 (0%)	
Concerns about tablets	0 (0%)	1 (0%)	
Doctor advice	3 (1%)	4 (2%)	
Withdrew consent	5 (2%)	6 (3%)	
Participants wishes	1 (0%)	2 (1%)	
Subtotal: Any other reason	11 (5%)	15 (7%)	0.54
Total: stopped for any reason	33 (16%)	34 (16%)	1.00

Supplementary Table 2: Effect of allocation to sacubitril/valsartan versus irebsartan on rate of change in estimated glomerular filtration rate

Time period	No. with value*	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	p value
Randomisation to 12 months	409	-0.22 (0.03)	-0.25 (0.03)	0.42
Randomisation to 3 months	410	-0.68 (0.12)	-0.61 (0.10)	0.63
3 months to 12 months	409	-0.11 (0.05)	-0.16 (0.04)	0.44

CKD-EPI=Chronic kidney disease Epidemiology Collaboration. eGFR=estimated glomerular filtration rate. Central creatinine measurements were only available at randomisation, 3, 6 and 12 months so local creatinine measurements were used at 1 and 9 months, after correction for the mean bias observed between local and central creatinine values. *Missing values of eGFR were imputed with the use of multiple imputation and participants with the most poorly fitting slopes (defined as participants with the mean deviation from their own fitted slope in the top 1% of the distribution [of mean deviations across all participants]) were excluded.

Supplementary Table 3: Associations between baseline characteristics and sacubitril/valsartan metabolite values at the 3 month visit

	Sacubitril		Sacubitrilat		Valsartan	
Characteristic	**Percentage change (95% CI)**	**p value**	**Absolute change in ng/mL (95% CI)**	**p value**	**Percentage change (95% CI)**	**p value**
Age, per decade higher	22% (1 to 48%)	0.04	889 (-30 to 1808)	0.06	14% (-2 to 33%)	0.09
Race*		0.78		0.71		0.22
Black	-60% (-95 to 252%)		-4856 (-15440 to 5729)		-63% (-94 to 112%)	
Other	-44% (-89 to 175%)		539 (-7208 to 8286)		5% (-71 to 276%)	
South Asian	7% (-71 to 297%)		1947 (-3684 to 7578)		-59% (-84 to 5%)	
Sex†	-25% (-67 to 71%)	0.48	-4413 (-8444 to -381)	0.03	-37% (-67 to 23%)	0.18
Body surface area, per 0.1 m² higher	-32% (-64 to 31%)	0.25	-3327 (-6500 to -154)	0.04	-23% (-54 to 31%)	0.34
Weight, per 5 kg higher	20% (-20 to 78%)	0.37	1915 (-25 to 3854)	0.05	13% (-18 to 56%)	0.44
mGFR (unadjusted for BSA), per 10 mL/min/1.73m² lower	-17% (-32 to 1%)	0.06	1485 (572 to 2397)	0.002	-3% (-17 to 13%)	0.65
Log albumin:creatinine ratio, per 5-fold increase	-7% (-24 to 13%)	0.44	-622 (-1590 to 346)	0.21	-14% (-27 to 1%)	0.07

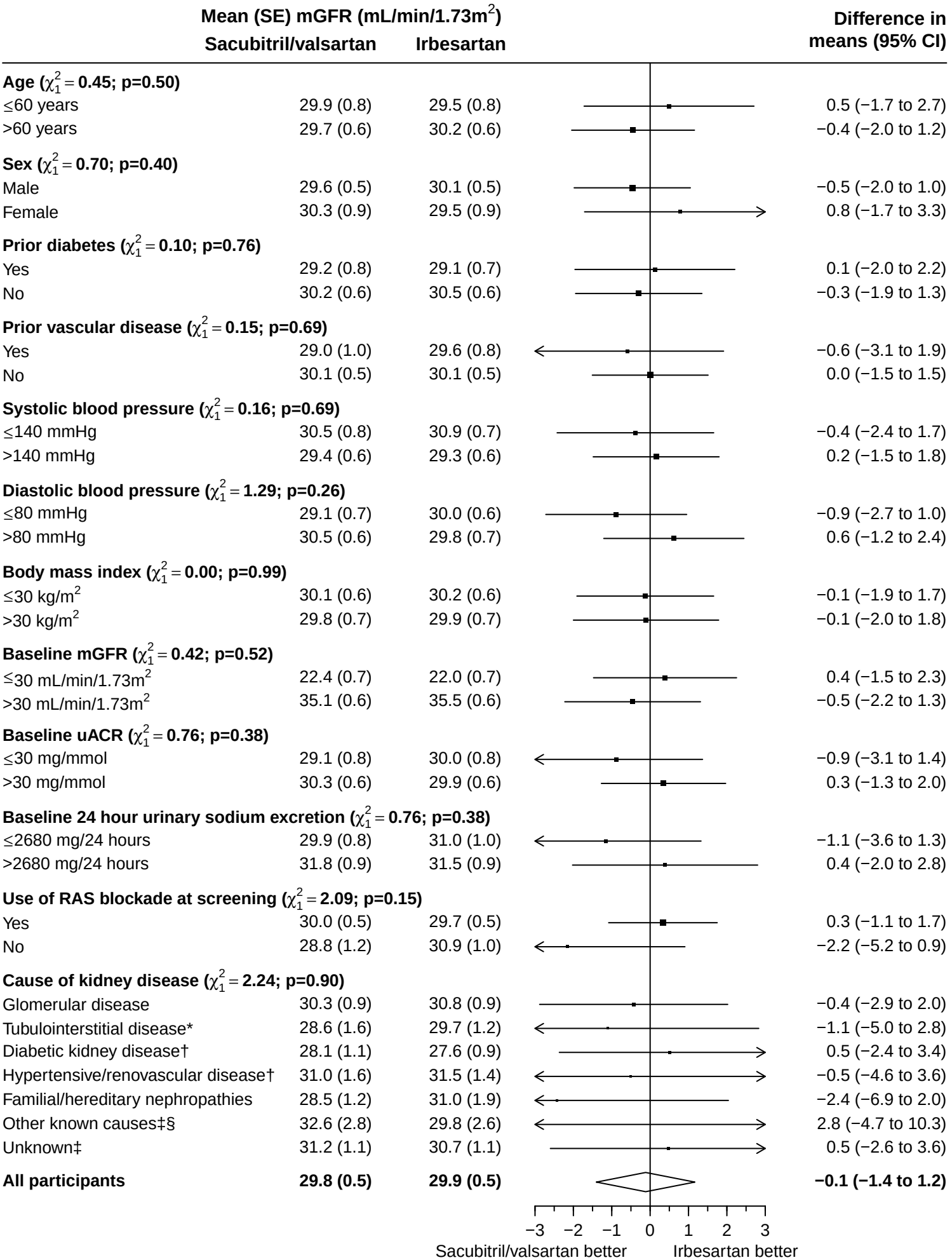
mGFR=measured glomerular filtration rate. BSA=body surface area. Models adjusted for all characteristics shown in table and additionally for time since last dose. *White ethnicity used as reference category. Race was not prespecified for inclusion in the models. †Males used as reference category. Values for sacubitril and valsartan were log tranformed due to skewed distributions.

Supplementary Table 4: Effect of allocation to sacubitril/valsartan versus irbesartan on serious adverse events and non-serious adverse reactions

	Sacubitril/valsartan (n=207)	Irbesartan (n=207)	Rate ratio (95% CI)	p value
Any fatal serious adverse event	1 (0.5%)	1 (0.5%)		
Non-fatal serious adverse events				
Angiooedema	1 (0.5%)	0 (0.0%)		
Hypotension	1 (0.5%)	1 (0.5%)		
Dialysis	2 (1.0%)	3 (1.4%)		
Other non-fatal SAEs (by MedDRA System, Organ, Class [SOC] category)				
Respiratory, thoracic and mediastinal disorders	6 (2.9%)	6 (2.9%)		
Infection and infestations	16 (7.7%)	15 (7.2%)		
Blood and lymphatics system	2 (1.0%)	2 (1.0%)		
Cardiac disorders	6 (2.9%)	5 (2.4%)		
Gastrointestinal disorders	5 (2.4%)	6 (2.9%)		
Metabolism and nutrition disorders				
Diabetes/glucose	3 (1.4%)	1 (0.5%)		
Other metabolism/nutrition	7 (3.4%)	6 (2.9%)		
Cancer	4 (1.9%)	5 (2.4%)		
Neoplasms benign, malignant and unspecified (incl. cysts and polyps)	2 (1.0%)	3 (1.4%)		
Nervous system disorders	3 (1.4%)	3 (1.4%)		
Renal and urinary disorders	10 (4.8%)	5 (2.4%)		
Other medical	30 (14.5%)	29 (14.0%)		
Investigations	8 (3.9%)	13 (6.3%)		
Surgical and medical procedures (excluding dialysis)	18 (8.7%)	14 (6.8%)		
Miscellaneous medical*	13 (6.3%)	8 (3.9%)		
Non-medical (including trauma)	7 (3.4%)	5 (2.4%)		
Total: Any non-fatal serious adverse event	61 (29.5%)	59 (28.5%)	1.07 (0.75-1.53)	0.70
Total: Any serious adverse event	61 (29.5%)	59 (28.5%)	1.07 (0.75-1.53)	0.70
Non-serious adverse reactions				
Hypotension	17 (8.2%)	7 (3.4%)	2.36 (1.06-5.26)	0.04
Hyperkalaemia	6 (2.9%)	1 (0.5%)	4.23 (0.96-18.61)	0.06
Acute kidney injury	3 (1.4%)	6 (2.9%)	0.51 (0.14-1.90)	0.32
Other NSAR (by MedDRA System, Organ, Class [SOC] category)				
Respiratory, thoracic and mediastinal disorders	4 (1.9%)	4 (1.9%)		
Gastrointestinal disorders	18 (8.7%)	10 (4.8%)		
Metabolism and nutrition disorders (excluding hyperkalaemia)	3 (1.4%)	1 (0.5%)		
Musculoskeletal and connective tissue disorders	6 (2.9%)	5 (2.4%)		
Nervous system disorders	20 (9.7%)	18 (8.7%)		
Renal and urinary disorders (excluding acute kidney injury)	2 (1.0%)	2 (1.0%)		
Reproductive system and breast disorders	2 (1.0%)	3 (1.4%)		
Skin and subcutaneous tissue disorders (excluding angiooedema)	18 (8.7%)	6 (2.9%)		
Other medical	6 (2.9%)	7 (3.4%)		
Investigations	3 (1.4%)	1 (0.5%)		
Miscellaneous medical**	8 (3.9%)	12 (5.8%)		
Total: Any non-serious adverse reaction* **	76 (36.7%)	58 (28.0%)	1.35 (0.96-1.90)	0.08

SAE=serious adverse event. NSAR=non-serious adverse reaction. *Made up of SOC categories: Ear disorders, Endocrine disorders, Eye disorders, Hepatobiliary disorders, Immune system disorders, Musculoskeletal and connectivetissue disorders, Psychriatric disorders, Reproductive system and breast disorders, Skin and subcutaneous tissue disorders (excluding angiooedema), Vascular disorders (excluding hypotension), Congenital, familialand genetic disorders, General disorders and administration site conditions, and Pregnancy, puerperium and perinatal conditions. **Made up of SOC categories: Infection and infestations, Blood and lymphatics system,Cardiac disorders, Ear disorders, Endocrine disorders, Eye disorders, Hepatobiliary disorders, Immune system disorders, Cancer, Neoplasms benign, malignant and unspecified (incl. cysts and polyps), Psychriatricdisorders, Skin and subcutaneous tissue disorders (excluding angiooedema), Vascular disorders (excluding hypotension), Surgical and medical procedures, Congenital, familial and genetic disorders, General disordersand administration site conditions, and Pregnancy, puerperium and perinatal conditions. ***Excluding angioodema.

Supplementary figure 1: Effect of allocation to sacubitril/valsartan on measured glomerular filtration rate at 12 months in different types of participants



mGFR = measured glomerular filtration rate. uACR=urinary albumin:creatinine ratio. RAS=renin−angiotensin system. *Includes obstructive renal diseases. †All considered 'Systemic diseases affecting the kidney' by the ERA−EDTA registry. ‡All considered 'Miscellaneous renal disorders' by the ERA−EDTA registry.§Includes other systemic kidney diseases.

Appendix 11:

Publication: review article of neprilysin Inhibition in CKD:

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Full Review

Neprilysin inhibition in chronic kidney disease

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ABSTRACT

Despite current practice, patients with chronic kidney disease (CKD) are at increased risk of progression to end-stage renal disease and cardiovascular events. Neprilysin inhibition (NEPi) is a new therapeutic strategy with potential to improve outcomes for patients with CKD. NEPi enhances the activity of natriuretic peptide systems leading to natriuresis, diuresis and inhibition of the renin–angiotensin system (RAS), which could act as a potentially beneficial counter-regulatory system in states of RAS activation such as chronic heart failure (HF) and CKD. Early NEPi drugs were combined with angiotensin-converting enzyme inhibitors but were associated with unacceptable rates of angioedema and, therefore, withdrawn. However, one such agent (omapatrilat) showed promise of NEP/RAS inhibition in treating CKD in animal models, producing greater reductions in proteinuria, glomerulosclerosis and tubulointerstitial fibrosis compared with isolated RAS inhibition. A new class of drug called angiotensin receptor neprilysin inhibitor (ARNi) has been developed. One such drug, LCZ696, has shown substantial benefits in trials in hypertension and HF. In CKD, HF is common due to a range of mechanisms including hypertension and structural heart disease (including left ventricular hypertrophy), suggesting that ARNi could benefit patients with CKD by both retarding the progression of CKD (hence delaying the need for renal replacement therapy) and reducing the risk of cardiovascular disease. LCZ696 is now being studied in a CKD population.

Keywords: cardiovascular disease, chronic kidney disease, heart failure, hypertension, neprilysin inhibition

INTRODUCTION

Patients with chronic kidney disease (CKD) face many hazards including increased risk of progression to end-stage renal disease (ESRD) and premature mortality from cardiovascular disease (CVD) [1, 2]. Whereas a minority of patients with CKD will reach ESRD, CVD is much more common. A variety of processes contribute to this excess risk including atherosclerosis, arteriosclerosis, hypertension, sympathetic hyperactivity and structural heart disease [including left ventricular (LV) hypertrophy], which may manifest clinically as heart failure (HF) [2]. As CKD progresses, the contribution of atherosclerosis becomes proportionally smaller and arteriosclerosis and structural heart disease predominate, potentially explaining the high incidence of sudden cardiac death in patients with advanced CKD [2]. The similarities in the manifestation of CVD observed in patients with advanced CKD and that in patients with HF raises the hypothesis that treatments proven to be effective in the HF population may also be beneficial in patients with advanced CKD. However, such patients have not been studied in randomized cardiological trials.

Randomized trials have shown that renin–angiotensin system (RAS) inhibitors [RASi; angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB)] reduce the risk of ESRD in patients with diabetic and non-diabetic proteinuric CKD [3–6]. In the general population, RASi reduce cardiovascular events, and meta-analyses suggest that the mechanism of this benefit is not simply blood pressure (BP) reduction [7, 8]. However, trials of RASi in patients with advanced CKD have not shown benefits on cardiovascular outcomes, although this may be because they were not large enough to do so [9].

Although dual ACEi/ARB therapy reduces albuminuria more than either agent alone, trials have shown that this does not translate into either cardiovascular benefit or additional

renal protection [10–13]. Indeed, in those trials, dual therapy was associated with increased risk of adverse effects including hyperkalaemia and acute kidney injury [11–13]. Similar outcomes were observed when RASi was combined with a direct renin inhibitor (aliskiren) as an alternative approach to dual RASi [14].

The lack of benefit associated with dual RAS blockade highlights the need for new therapeutic strategies in CKD. The natriuretic peptide (NP) system is a neurohormonal system that counter-regulates the RAS. Therefore, enhancing the activity of NPs may be beneficial in states of RAS activation, such as cardiovascular and kidney disease.

NP SYSTEM AND NEPRILYSIN

NPs are a family of three peptides that include atrial, brain and c-type NPs (ANP, BNP and CNP, respectively) [15]. ANP and BNP are predominantly synthesized and released from cardiac myocytes in response to atrial stretch due to raised venous pressure. ANP precursor expression in the kidney produces a subtype called urodilatin from distal tubular cells, whereas CNP is predominantly expressed in endothelial cells [15, 16]. All three NPs are formed as pre-pro-peptides and undergo several cleavage steps to form active peptides. NPs exert physiological effects via NP receptors (NPRs). ANP and BNP act via NPR-A (guanylate cyclase-A) and CNP via NPR-B (guanylate cyclase-B) [17]. These receptors are coupled to cyclic guanosine monophosphate (cGMP)-dependent signalling (Figure 1) [15–17].

ANP and BNP have a range of renal and cardiovascular effects contributing to natriuresis, diuresis and BP regulation [16, 17]. CNP is a vasoactive peptide with marked cardiovascular effects but minimal renal actions [16, 17]. Both ANP and urodilatin regulate renal sodium and water excretion by inhibition of angiotensin II- and aldosterone-dependent sodium and water reabsorption and inhibition of antidiuretic hormone [17]. Natriuresis results from afferent arteriolar vasodilatation and efferent arteriolar vasoconstriction, increasing renal vascular resistance and glomerular filtration. ANP also causes relaxation of mesangial cells, further increasing the capillary surface area for filtration and hence diuresis [18]. In addition, ANP inhibits endothelin production, proliferation of smooth muscle cells and myocardial hypertrophy [17, 18].

Animal models lacking the proANP gene develop salt-sensitive hypertension [19]. Gene delivery of ANP to mice with salt-sensitive hypertension reduces BP, cardiac hypertrophy, stroke and renal injury [20, 21]. Recently, two single nucleotide polymorphisms rs5068 and rs1938358 in the ANP and BNP genes have been found to be associated with both increased levels of NT-proANP and NT-proBNP, respectively, and with lower BP and an improved metabolic profile [22]. These genetic data suggest that augmenting NP concentrations could lead to improved clinical outcomes.

Neprilysin

Neprilysin [also known as neutral endopeptidase (NEP)] is the key enzyme responsible for degradation of NPs [17]. NEP is a membrane-bound zinc-containing metalloproteinase with widespread tissue distribution including the brain, vascular endothelial cells, smooth muscle cells, cardiac myocytes and neutrophils, but has greatest abundance in the brush border of proximal renal tubular cells [16, 23]. NEP is also responsible for processing and catabolism of a range of other vasoactive peptides including bradykinin, substance P, angiotensin II and endothelin [23].

The broad range of potential therapeutic actions of NPs led to development of agents that inhibit NEP. Neprilysin inhibition (NEPi) results in potent natriuresis and vasodilation; in

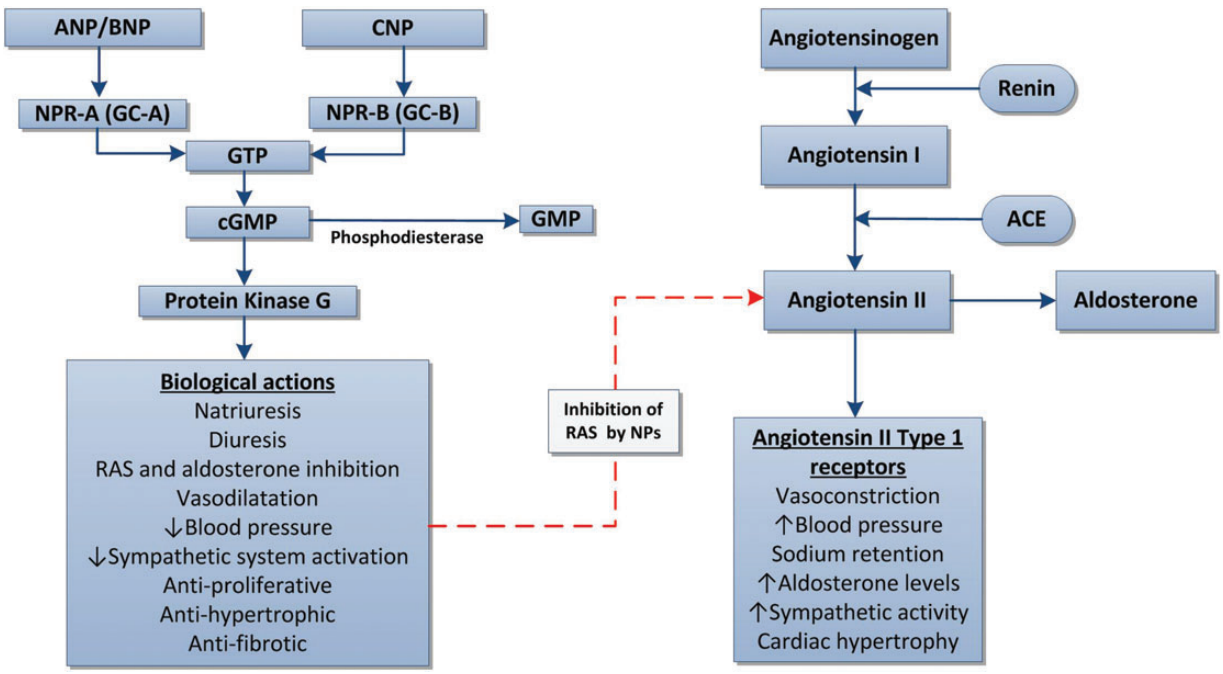

FIGURE 1: Mechanism of action of NPs [16, 17]. GTP, Guanosine-5′-triphosphate.

Table 1. VPIs produced and studied in humans

VPI	Situation studied	Year
MDL-100240	Healthy volunteers	2000
Sampatrilat	Hypertension	1998–99
Fasidotril	Hypertension	2000
Omapatrilat (BMS-186716)	Hypertension, HF and CVD	1999–2004

the kidney, this vasodilatory effect reduces intraglomerular pressure and proteinuria [24, 25]. Chronic isolated NEPi does not translate into clinically meaningful BP reductions as NEPi impairs breakdown of angiotensin II and any BP effects are offset by up-regulation of RAS and sympathetic nervous system activity. The beneficial renal and cardiovascular effects of NEPi are enhanced when combined with RASi and this has led to development of dual NEPi/RASi [16].

DUAL NEPi/ACEi (VASOPEPTIDASE INHIBITORS)

Dual NEPi/ACEi are also known as vasopeptidase inhibitors (VPIs). Many compounds have been produced and trialled in humans (Table 1). Omapatrilat was the most studied VPI.

Omapatrilat was well tolerated in studies of healthy volunteers among whom it significantly increased urinary excretion of ANP and cGMP (i.e. markers of NEPi). Omapatrilat also produced potent ACE inhibition with decreased levels of angiotensin II and reduced systemic BP. Renal effects of omapatrilat included marked increases in renal blood flow without associated change in glomerular filtration rate (GFR) and decreased filtration fraction. This haemodynamic profile could translate into renal protection and slower progression of CKD, as discussed further below [24, 25].

VPIs and angioedema

Despite the promising cardiorenal and neurohormonal findings seen with VPIs, omapatrilat was associated with excess rates of angioedema. In 723 patients with CVD, omapatrilat reduced BP but six cases of angioedema occurred [26]. In the Omapatrilat Cardiovascular Treatment versus Enalapril (OCTAVE) trial, angioedema occurred with greater severity and frequency with omapatrilat than enalapril [274/12 609 (2.17%) versus 86/12 557 (0.68%); relative risk 3.17; 95% confidence interval (95% CI) 2.52–4.12; $P < 0.005$] [27]. Two of the participants experienced airway compromise, one of whom required mechanical ventilation. The mechanism of angioedema was found to be related to increased bradykinin activity with combined NEPi and ACEi (described below).

Angioedema is an uncommon complication of ACEi therapy which is seen in 0.1–0.3% of treated patients and can occur at any interval after starting these drugs [28]. It can very rarely cause laryngeal oedema and asphyxiation leading to death [16]. ACE inhibitor-induced angioedema is thought to be mediated by decreased bradykinin breakdown resulting in increased bradykinin levels (Figure 2) [28, 29]. In an acute episode of angioedema, bradykinin concentrations can rise >10-fold [28].

Given the low incidence of angioedema associated with ACEi, it is thought that individuals are only affected if they have an additional risk factor, such as smoking (due to reduced NEP and dipeptidyl peptidase IV activity in smokers), black race (due to ACE gene polymorphisms) or hereditary angioedema (for example due to C1 inhibitor deficiency) [29, 30]. With ACE inhibition, bradykinin degradation becomes dependent on secondary enzymes (including NEP) for its breakdown and hence combined NEPi/ACEi had an additive effect on bradykinin levels. Following the results of the OCTAVE trial, the Food and Drug Administration review board did not approve omapatrilat and it was withdrawn from development by the manufacturer [16].

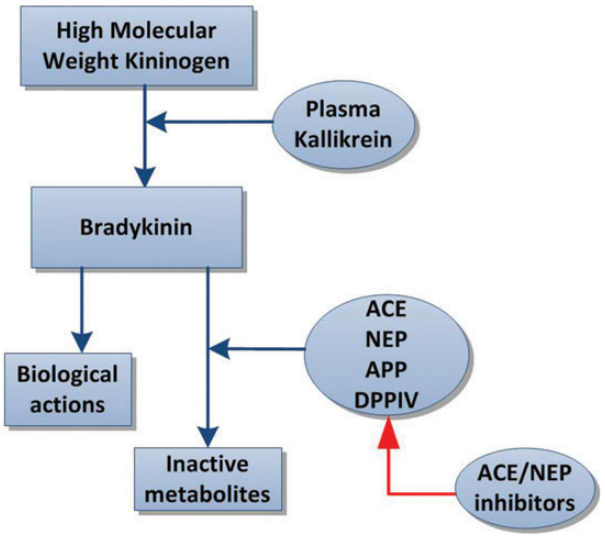

FIGURE 2: Mechanism of bradykinin action and inactivation by neprilysin.

DUAL NEP/ARB INHIBITION

Whilst ACEi induce RAS blockade by inhibiting the conversion of angiotensin I to angiotensin II, angiotensin II receptor blockers (ARBs) elicit similar effects by blocking the activation of angiotensin II Type 1 receptors by angiotensin II. However, ARBs have minimal effect on bradykinin activity and, therefore, are much less likely to cause angioedema. This led to the development of dual-acting angiotensin receptor neprilysin inhibitors (ARNi), which combine the beneficial effects of ARBs and NEPi without excess risk of angioedema (Figure 3). LCZ696 was the first ARNi to be developed. It combines two drugs: an ARB moiety (valsartan) and an NEP inhibitor prodrug (AHU377) in a 1:1 molar complex. Oral administration of LCZ696 delivers systemic exposure to the two separate moieties. AHU377 has a relatively short half-life and undergoes further rapid conversion by enzymatic cleavage of its ethyl ester to form the active NEPi compound, LBQ657 [31, 32].

In studies of healthy volunteers, AHU377 reached peak plasma concentrations in 0.5–1.1 h and the active moiety LBQ657 in 1.8–3.5 h [32]. LCZ696 was associated with increases in plasma cGMP, renin and angiotensin II levels. Systemic exposure to valsartan following dosing with LCZ696 demonstrated bioequivalence [e.g. 400 mg LCZ696 (maximum

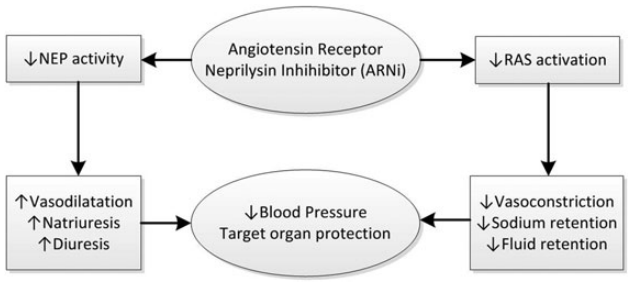

FIGURE 3: Mechanism of action of ARNi.

dose) is equivalent to 320 mg of valsartan] [32]. The drug was well tolerated in these participants [32, 33].

NEPi IN HYPERTENSION

NEPi was originally studied using VPIs in a range of animal models of hypertension including salt-sensitive hypertension, stroke-prone spontaneous hypertensive rats and renovascular hypertension.

In the OCTAVE trial involving 25 302 hypertensive patients [27], compared with enalapril, at 8 weeks omapatrilat reduced systolic BP (SBP) by 3.6 mmHg (95% CI 2.6–4.6; P<0.001) and by 24 weeks fewer participants required adjunctive antihypertensive therapies (19 versus 27%, P < 0.001) [27].

A trial of 1328 hypertensive patients compared increasing doses of LCZ696 (100, 200 and 400 mg), valsartan (80, 160 and 320 mg), AHU377 (200 mg) or placebo [33]. The primary end point was mean change from baseline in mean sitting diastolic BP (DBP) between LCZ696 and valsartan during the 8-week treatment period. At the end of 8 weeks, the three LCZ696 doses had superior DBP lowering (mean reduction 2.17 mmHg; 95% CI 1.06–3.28; P < 0.0001) compared with the appropriate comparator dose of valsartan [33]. Single-dose pairwise comparisons showed that each dose of LCZ696 had greater SBP and DBP lowering than its equivalent dose of valsartan, and that the proportional reduction in SBP and of DBP increased with increasing LCZ696 dosage [results for mean change in SBP and DBP for LCZ696 (LCZ) 400 mg versus valsartan (Val) 320 mg are shown in Figure 4].

Plasma ANP and cGMP levels increased significantly with LCZ696. LCZ696 reduced albuminuria more than placebo, but not more than the equivalent dose of valsartan [33]. However, baseline albuminuria was low (geometric mean between 1.1 and 1.5 mg/mmol in all treatment groups). LCZ696 was well tolerated and no cases of angioedema occurred [33].

A recent trial has demonstrated similar efficacy in Asian patients (from Japan, China, Korea, Taiwan and Thailand) with hypertension, who are generally less responsive to isolated RASi [34]. The Prospective comparison of Angiotensin Receptor neprilysin inhibitor with Angiotensin receptor blocker MEasuring arterial sTiffness in the eldERly (PARAMETER) study is assessing the efficacy of LCZ696 versus olmesartan on central aortic haemodynamics and aortic stiffness in 432 patients (aged >60 years) [35]. The results are expected in 2015.

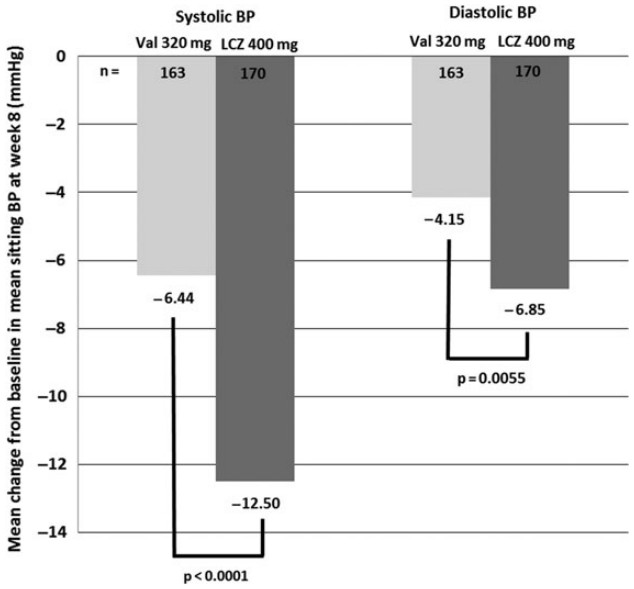

FIGURE 4: Difference in mean sitting SBP and DBP at week 8 (mmHg) [33].

NEPi IN HEART FAILURE

In early HF, NP levels increase to counteract salt and water retention. Over time the effects of NPs are negated by up-regulation of neurohormonal pathways, including RAS and the sympathetic nervous system, which cause further salt and water retention. Increasing levels of NPs with NEPi may help counteract up-regulation of these pathogenic pathways, when combined with RAS blockade.

The Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events (OVERTURE) trial randomized 5770 patients with New York Heart Association (NYHA) Classes II–IV HF to either omapatrilat or enalapril [36]. Non-significantly fewer patients treated with omapatrilat died or were hospitalized for HF compared with enalapril [914/2886 (32%) versus 973/2884 (34%); HR 0.94; 95% CI 0.86–1.03; P = 0.187] [36]. Angioedema was again more frequent with omapatrilat (0.8%) than enalapril (0.5%) but was less severe than in other trials [36].

The Prospective comparison of ARNi with ARB on Management Of heart failUre with preserved ejectioN fracTion (PARAMOUNT) trial randomized 301 patients with HF with preserved ejection fraction (HFpEF) to maximum tolerated daily doses of LCZ696 or valsartan [37]. The primary end point was change in NT-proBNP (as a marker of LV wall stress) from baseline to 12 weeks. NT-proBNP is a useful marker to study as it is not degraded by NEP, so any changes in NT-proBNP levels can still be used to assess disease severity in HF with NEPi [38]. In PARAMOUNT, greater reductions in NT-proBNP were seen with LCZ696 (ratio of change from baseline to 12 weeks 0.77; 95% CI 0.64–0.92; P = 0.005), in addition to improved NYHA class, BP and left atrial size. The drug was well tolerated, and although one case of angioedema occurred, it did not require hospitalization [37].

The Prospective comparison of ARNi with ACEi to Determine Impact on Global Mortality and morbidity in Heart Failure trial (PARADIGM-HF), the largest ever trial in HF with reduced ejection fraction (NYHA Classes II–IV) randomized 8436 patients to maximum daily tolerated doses of LCZ696 or enalapril [31, 39]. The primary outcome was a composite of time to first occurrence of either cardiovascular death or hospitalization for HF. Mean BP (mmHg) at baseline was 121/74 and LV ejection fraction 29% [39]. Mean serum creatinine at baseline was 99 μmol/L [mean estimated GFR (eGFR) 68 mL/min/1.73 m^2] and 37% of participants had an eGFR <60 mL/min/1.73 m^2 at enrolment [39].

The trial was closed early on the recommendation of the Data Monitoring Committee, having met the primary end point with overwhelming efficacy in favour of LCZ696 [40]. The full results of the trial are expected in the summer of 2014.

The Prospective comparison of ARni with Arb Global Outcomes in heart failure with preserved ejection fraction (PARAGON-HF) will soon start recruiting about 4300 patients with HFpEF and compare LCZ696 with valsartan. The primary outcome will be a composite of cardiovascular death and total (first and recurrent) hospitalizations for HF [38].

NEPi IN CKD

The evidence for a potential role of NEPi in CKD comes from the study of NEPi in animal models of renal disease and the results of renal outcomes from trials in HF. However, no large-scale human trials have been conducted with this class of agents in a CKD cohort.

In an animal model of hypertension, long-term administration of omapatrilat led to dose-dependent reductions in BP and proteinuria that halted progression of glomerulosclerosis, tubulointerstitial fibrosis and renal injury [24]. In a 5/6 nephrectomy model, the anti-hypertensive and renoprotective effects of the VPI AVE7688 were compared with enalapril. Treatment was started once proteinuria and hypertension developed. AVE7688 greatly reduced proteinuria, glomerulosclerosis and tubulointerstitial fibrosis on renal biopsy [23]. Similar findings have also been observed in models of diabetic nephropathy [41]. AVE7688 increased renal synthesis of nitric oxide and decreased synthesis of endothelin-1 with reduced renal vasoconstriction and increased tubular ANP release [23]. In another 5/6 nephrectomy model, omapatrilat was administered at various time points following surgery [25]. Micropuncture studies demonstrated that omapatrilat led to greater reductions in SBP and capillary glomerular pressure [25]. The study also demonstrated reduced proteinuria and greater protection from renal injury with reduced glomerulosclerosis and delayed progression of renal disease with omapatrilat compared with ACEi-alone, which is likely to result from the effect on glomerular capillary pressure [25].

Candoxatrilat, an isolated NEPi, was compared with placebo in 24 patients with normal, moderately or severely reduced GFR in a cross-over study [42]. Compared with the placebo infusion, plasma ANP and urinary cGMP rose significantly after a 100-mg intravenous bolus of candoxatrilat. A marked natriuresis and diuresis occurred in all groups without changes in GFR or systemic BP [42].

In the Inhibition of Metallo Protease by Omapatrilat in a Randomized Exercise and Symptoms Study of Heart Failure (IMPRESS) trial (comparing omapatrilat with lisinopril), creatinine levels were reported as being raised more frequently in patients treated with lisinopril than omapatrilat (6.1 versus 1.8%, respectively; P = 0.009) [43]. Similarly, in the OVERTURE trial, worsening renal impairment occurred less frequently with omapatrilat (6.8 versus 10.1% with enalapril), despite including patients with moderate renal impairment (eligibility required serum creatinine <221 μmol/L at baseline) [36]. Over 36 weeks of follow-up of the PARAMOUNT trial (comparing LCZ696 with valsartan), eGFR declined to a lesser degree in the LCZ696 group (LCZ696, −1.6 mL/min/1.73 m^2 versus valsartan, −5.2 mL/min/1.73 m^2; P = 0.007) [37]. However, albuminuria increased by 1 mg/mmol with LCZ696 compared with no change with valsartan (P = 0.02), but was very low at baseline [mean urine albumin:creatinine ratio (ACR) 2.0 mg/mmol] [37]. The PARADIGM-HF protocol includes renal-specific secondary end points: time to the composite of (i) 50% decline in eGFR relative to baseline, (ii) >30 mL/min/1.73 m^2 decline in eGFR relative to baseline eGFR of <60 mL/min/1.73 m^2 or (iii) progression to ESRD [31].

These studies highlight the potential advantages of combined NEPi/RASi in slowing the progression of CKD. However, the current data are indirect as they are based on animal models or HF populations. The UK Heart And Renal Protection III (UK HARP-III) trial (ISRCTN11958993) will compare LCZ696 against irbesartan in 360 patients with proteinuric CKD (urine ACR >20 mg/mmol and eGFR ≥20 <60 mL/min/1.73 m^2). The trial will be the first test of an ARNi in a proteinuric population, and will assess the short-term safety and efficacy of LCZ696 in CKD with a primary outcome of the difference in change in measured GFR from baseline to 6 months between the two arms.

CONCLUSION

NPs act as a potentially beneficial counter-regulatory system in states of excess RAS activation such as seen in hypertension, HF and CKD. In hypertension and HF, inhibition of neprilysin with LCZ696 has been shown to provide substantial clinical benefit. For patients with CKD, NEPi could be beneficial for two reasons: first, it may reduce the risk of CVD; second, it may retard the progression of CKD itself and delay the need for renal replacement therapy. A large randomized trial of an ARNi in a CKD population will be required to investigate this potential, but—if positive—such a trial would have a substantial impact on clinical practice.

CONFLICT OF INTEREST STATEMENT

The UK HARP-III trial has been funded by Novartis. It will be conducted and interpreted independently of the principal funder. The Clinical Trials Service Unit and Epidemiological Studies Unit, which is part of the University of Oxford, has a staff policy of not accepting honoraria or consultancy fees.

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REVIEW

Chronic kidney disease, heart failure and neprilysin inhibition

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ABSTRACT

Patients with chronic kidney disease are at increased risk of cardiovascular disease and this often manifests clinically like heart failure. Conversely, patients with heart failure frequently have reduced kidney function. The links between the kidneys and cardiovascular system are being elucidated, with blood pressure being a key risk factor. Patients with heart failure have benefitted from many trials which have now established a strong evidence based on which to base management. However, patients with advanced kidney disease have often been excluded from these trials. Nevertheless, there is little evidence that the benefits of such treatments are modified by the presence or absence of kidney disease, but more direct evidence among patients with advanced kidney disease is required. Neprilysin inhibition is the most recent treatment to be shown to improve outcomes among patients with heart failure. The UK HARP-III trial assessed whether neprilysin inhibition improved kidney function in the short- to medium-term and its effects on cardiovascular biomarkers. Although no effect (compared to irbesartan control) was found on kidney function, allocation to neprilysin inhibition (sacubitril/valsartan) did reduce cardiac biomarkers more than irbesartan, suggesting that this treatment might improve cardiovascular outcomes in this population. Larger clinical outcomes trials are needed to test this hypothesis.

Keywords: blood pressure, cardiovascular, CKD, heart failure, renin-angiotensin system

CKD AND STRUCTURAL HEART DISEASE ARE CLOSELY ASSOCIATED

Chronic kidney disease (CKD) and heart failure (HF) frequently coexist and both are associated with high morbidity and mortality [1, 2]. Numerous studies have shown that there is an inverse association between kidney function and cardiovascular risk [3, 4]. Structural heart disease, which may manifest clinically as HF, is a leading cause of cardiovascular disease in CKD patients and its prevalence increases with declining kidney function [2, 5]. A cross-sectional echocardiographic observational study reported an increasing prevalence of left ventricular hypertrophy (LVH) with decreasing estimated glomerular filtration rate (eGFR) (from 32% among patients with eGFR $\geq$60 mL/min/1.73 m^2 to 75% among patients with eGFR $<$30 mL/min/1.73 m^2) [6, 7]. Studies using cardiac magnetic resonance imaging with gadolinium enhancement have found that diffuse late gadolinium enhancement is associated with the degree of LVH [8] and indicates myocyte disarray and interstitial fibrosis histologically [9]. Although overt systolic dysfunction is not common (affecting only 8% of patients in the above cross-sectional echocardiographic study) and not clearly associated with kidney function [7], more subtle disturbances in ventricular function (such as reduced left ventricular deformation, early myocardial relaxation velocity or reduction in global longitudinal strain that may contribute to diastolic dysfunction) are more common and are present even in the early stages of CKD [10, 11]. These abnormalities provide the anatomical substrate for the excess risk of symptomatic HF, arrhythmia and sudden cardiac death observed among patients with advanced CKD. Conversely, in large HF registries, 20–68% of patients with HF have moderate to severe kidney disease [1]. The presence of CKD is associated with poor prognosis in HF and can be used to stratify the risk of patients with HF [6, 12, 13].

PATHOPHYSIOLOGY OF HF IN CKD

The pathophysiological relationship between the heart and the kidneys involves many different pathways. CKD may disturb homoeostasis in ways that may be directly damaging to the cardiovascular system [i.e. 'direct' risk factors such as high blood pressure (BP) or vascular calcification] or the kidneys and

circulation may both be subject to 'indirect' risk factors (e.g. diabetes mellitus and smoking). In addition, HF may worsen CKD by decreasing renal perfusion, causing renal venous congestion and activation of the sympathetic nervous system and renin–angiotensin–aldosterone system (RAS, which may in turn cause inflammation and oxidative stress). Treatment for HF in CKD can be divided into two broad types: (i) treatments that intervene on pathophysiological links between CKD and HF to prevent HF and (ii) treatments known to improve prognosis in established HF among people without CKD.

TREATMENT TO PREVENT HF IN CKD

CKD is commonly associated with high BP, due to salt and water retention, activation of the sympathetic nervous and other neurohormonal systems and accumulation of endogenous vasopressors [14]. Studies of living kidney donors suggest that reducing GFR by 10 mL/min as a consequence of donor nephrectomy leads to a 5 mmHg increase in systolic BP [15]. BP is positively associated with the risk of death from HF [16] and randomized trials have demonstrated that this association is causal [17]. Meta-analysis of all the major BP-lowering trials has shown that a 10 mmHg reduction in systolic BP lowers the risk of HF by 28% [95% confidence interval (CI) 22–33] [18]. Most classes of antihypertensive treatments have similar effects, with the exception of calcium channel blockers (which may have a smaller benefit) and diuretics (which may have a larger benefit) [18]. A subgroup analysis within this meta-analysis (which included 13 trials involving nearly 38 000 participants, of whom 6000 had CKD) suggested that the effect of BP lowering on HF was larger among patients without CKD [relative risk (RR) 0.48 (95% CI 0.38–0.62)] than among patients with CKD [RR 0.95 (95% CI 0.70–1.04); P for interaction <0.001] [18]. Nevertheless, the benefits of lowering BP on other cardiovascular outcomes remain clear even among patients with CKD.

Anaemia is a well-recognized complication of CKD and has been proposed as a direct cause of HF in patients with CKD following observational and non-randomized interventional studies, suggesting that anaemia is associated with LVH and correcting the anaemia reverses the LVH [19, 20]. However, randomized trials have shown that full or partial correction of anaemia with erythropoiesis-stimulating agents (ESAs) does not reduce left ventricular mass nor the risk of HF and may even increase the risk of other cardiovascular outcomes such as stroke [21].

Reducing parathyroid hormone concentrations with calcimimetic therapy might reduce the risk of non-atherosclerotic cardiovascular events (such as HF) among haemodialysis patients [22, 23]. Such treatment also reduces fibroblast growth factor 23 (FGF23; see below). Unfortunately, the randomized data on other interventions that target CKD-specific mechanisms of HF are much less robust. For example, although there is evidence that hyperphosphataemia (i) can cause vascular smooth muscle cells to adopt an osteoblastic phenotype and cause vascular calcification (which in turn increases cardiac afterload) [24] and (ii) is associated with LVH [25], no sufficiently large trials of phosphate reduction have been conducted to elucidate whether these associations are causal. Although FGF23 has been found to induce LVH after direct intracardiac injection in mice [26], the totality of the observational evidence does not suggest that FGF23 is a cause of cardiovascular disease (and no trials of FGF23 reduction in CKD exist) [27].

TREATMENT TO IMPROVE PROGNOSIS IN ESTABLISHED HF IN THE GENERAL POPULATION

The main objectives of HF therapy in CKD (as well as in non-CKD) patients are to decrease the preload and afterload and to reduce LVH, treat myocardial ischaemia and inhibit neurohumoral hyperactivity, especially the sympathetic nervous system and RAS [28]. However, the optimum treatment of HF in patients with CKD remains unclear, as there is little direct evidence to support any recommendations. Most of the pivotal randomized trials that guide the management of HF define CKD as a baseline eGFR <60 mL/min/1.73 m^2 but have excluded patients with more advanced stages of CKD (i.e. eGFR <30 mL/min/1.73 m^2).

Many pharmacological and device treatments are recommended for HF with reduced ejection fraction (HFrEF) [29]. The mainstays of such treatment are angiotensin-converting enzyme inhibitors (ACEis) and β-blockers. The largest trial of ACEis in HFrEF was Studies of Left Ventricular Dysfunction (SOLVD)-Treatment, which compared enalapril 10 mg twice daily with placebo among 2569 patients with HFrEF and demonstrated a 16% (95% CI 5–26) reduction in mortality (primary outcome) [30]. This effect was similar in patients with and without CKD [31]. Similarly, in the four large trials of β-blockers in HFrEF, there was no good evidence that the benefits of β-blocker therapy were modified by baseline kidney function. The results of these trials (and their published effects by baseline kidney function) are summarized in Table 1.

For patients with HFrEF [with a left ventricular ejection fraction (LVEF) <35%] who remain symptomatic after optimization of ACEi and β-blocker therapy, guidelines recommend a mineralcorticoid receptor antagonist (MRA). This recommendation follows two large trials (see Table 1). Again, the effect of treatment on the primary outcome was not modified by baseline kidney function. However, these trials highlight the importance of safety as a consideration in the treatment of patients with CKD. Patients with CKD are at higher risk of hyperkalaemia (due to the reduced ability of their kidneys to excrete potassium), which is associated with an increased risk of hospitalization and death [43]. The trials had stringent monitoring of serum potassium and developed criteria for reducing the dose or stopping the MRA, such that there was no excess death due to hyperkalaemia in the trials. The importance of such monitoring is highlighted by population-based studies, which demonstrate increased rates of hospitalization for hyperkalaemia since the publication of these trials [44]. Device therapies [implantable cardioverter defibrillators (ICDs) and cardiac resynchronization therapy

Table 1. Effect of kidney function on the efficacy of established treatments for chronic HFrEF

Trial (ref)	Intervention (sample size)	Main eligibility criteria	Follow-up (years)	Primary outcome	Overall treatment effect (95% CI)	CKD subgroups (eGFR, mL/min/1.73 m^2)	Treatment effect in CKD	P for treatment × CKD interaction
ACEi								
SOLVD-TREATMENT [31]	Enalapril versus placebo ($n = 2569$)	LVEF ≤35%; NYHA I–IV; creatinine <177 µmol/L	3.5	All-cause mortality	0.84 (0.74–0.95)	≥60 ($n = 1466$) <60 ($n = 1036$)	0.82 (0.69–0.98) 0.88 (0.73–1.06)	0.62
β-blocker								
CIBIS-II [32]	Bisoprolol versus placebo ($n = 2647$)	LVEF ≤35%; NYHA III–IV; creatinine <300 µmol/L	1.3	All-cause mortality	0.66 (0.54–0.81)	<45 ($n = 450$) ≥45 <60 ($n = 669$) ≥60 <75 ($n = 640$) >75 ($n = 863$)	0.71 (0.48–1.05) 0.69 (0.46–1.04) 0.53 (0.34–0.82) 0.64 (0.42–0.99)	0.81
MERIT-HF [33, 34]	Metoprolol versus placebo ($n = 3991$)	LVEF ≤40%; NYHA II–IV; 'significant' kidney disease	1	All-cause mortality	0.66 (0.53–0.81)	<45 ($n = 493$) ≥45–≤60 ($n = 976$) >60 ($n = 2496$)	0.41 (0.25–0.68) 0.68 (0.45–1.02) 0.71 (0.54–0.95)	0.095
SENIORS [35, 36]	Nebivolol versus placebo ($n = 2128$)	LVEF <35% or hospitilization for decompensated HF; NYHA II–IV; creatinine <250 µmol/L	1.75	All-cause mortality or CV hospital admission	0.86 (0.74–0.99)	<55.5 ($n = 704$) 55.5–72.8 ($n = 704$) >72.8 ($n = 704$)	0.84 (0.67–1.07) 0.79 (0.60–1.04) 0.86 (0.65–1.14)	0.442
Mineralocorticoid receptor antagonist								
RALES [37, 38]	Spironolactone versus placebo ($n = 1663$)	LVEF <35%; NYHA III–IV; creatinine ≤221 µmol/L	2	All-cause mortality	0.70 (0.60–0.82)	<60 ($n = 792$) ≥60 ($n = 866$)	0.68 (0.56–0.84) 0.71 (0.57–0.90)	N/A
EMPHASIS-HF [39]	Eplerenone versus placebo ($n = 2737$)	LVEF ≤35%; NYHA II; eGFR ≥30 mL/min/1.73 m^2	1.75	CV death or hospitalization for HF	0.63 (0.54–0.74)	<60 ($n = 912$) ≥60 ($n = 1821$)	N/A N/A	0.50
Angiotensin receptor neprilysin inhibitor								
PARADIGM-HF [40]	Sacubitril/valsartan versus enalapril ($n = 8442$)	LVEF ≤40%; NYHA II–IV; eGFR ≥30 mL/min/1.73 m^2	2.25	CV death or hospitalization for HF	0.80 (0.73–0.87)	<60 ($n = 3061$) ≥60 ($n = 5338$)	N/A N/A	0.91
ICD								
MADIT II [41]	Prophylactic ICD versus conventional medical therapy ($n = 1232$)	LVEF ≤30%; NYHA III; eGFR ≥15 mL/min/1.73 m^2	2.67	All-cause mortality	0.69 (0.51–0.93)	<35 ($n = 80$) 35–59 ($n = 387$) ≥60 ($n = 756$)	1.09 (0.49–2.43) 0.74 (0.48–1.15) 0.66 (0.43–1.02)	0.29
CRT								
CARE-HF [42]	CRT versus conventional medical therapy ($n = 813$)	LVEF ≤35%; NYHA III–IV;	1.5	Death from any cause or unplanned hospitalization for a major CV event	0.63 (0.51–0.77)	<60 ($n = 369$) ≥60 ($n = 370$)	0.67 (0.50–0.89) 0.57 (0.40–0.80)	N/A

Data extracted from large trials where subgroup analysis by kidney function is available. NYHA, New York Heart Association; CV, cardiovascular; N/A, not available.

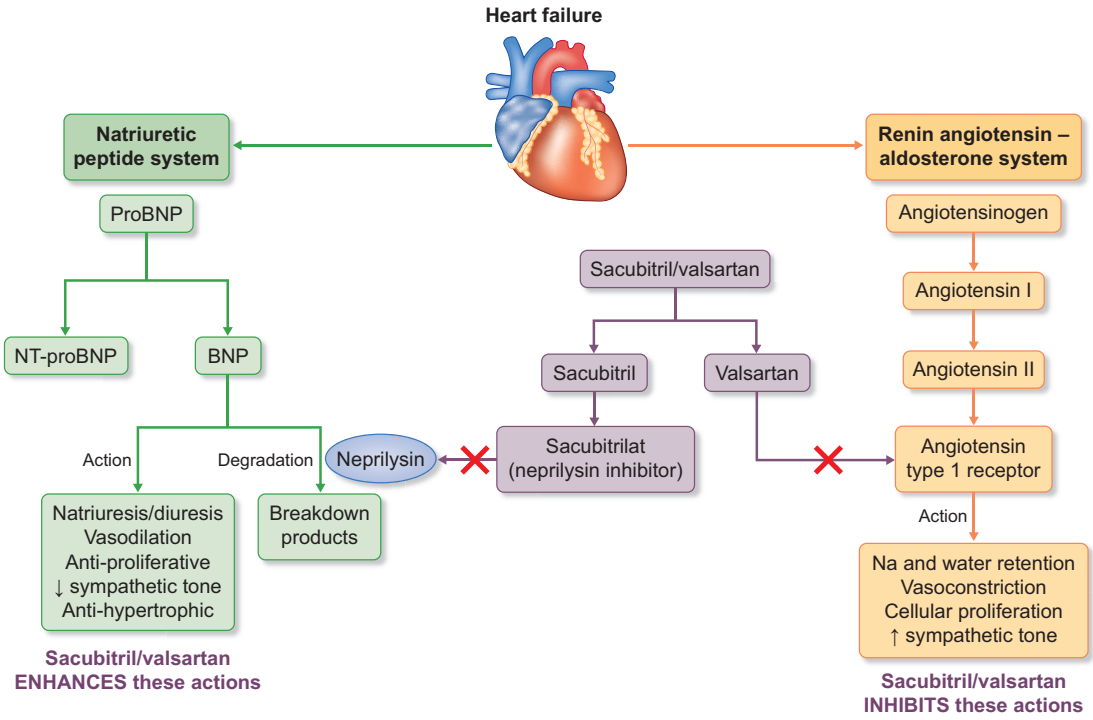

FIGURE 1: Effects of sacubitril/valsartan on vasoactive peptides.

(CRT)] also improve prognosis in selected patients with HFrEF). A meta-analysis of the trials of ICDs has raised the hypothesis that worse kidney function might attenuate the benefit of these devices [45], but this is not the case for CRT devices. Intravenous iron has been shown to improve functional capacity among patients with HFrEF and results of clinical outcomes trials are needed [46]. Indeed, the PIVOTAL trial among haemodialysis patients suggests that intravenous iron may reduce cardiovascular morbidity in this population [47]. This finding may alter the interpretation of the placebo-controlled ESA trials in which participants allocated to placebo received more iron.

However, as noted above, few patients with CKD have HFrEF, whereas structural substrates for diastolic dysfunction are common among patients with CKD. In contrast with HFrEF, no treatment has yet demonstrated convincing benefit (in terms of morbidity and mortality) in patients with HF with moderately reduced EF (HFmrEF: LVEF ≥40–<50%) or HF with preserved EF (HFpEF: LVEF ≥50%). The Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist trial tested spironolactone (15–45 mg daily) versus placebo in 3445 patients with LVEF ≥45% and observed a non-significant 11% (95% CI −4–23) reduction in the primary outcome of cardiovascular death, aborted cardiac arrest or hospitalization for HF [37]. There was again no modification of the treatment effect by baseline kidney function. However, *post hoc* analyses have suggested that patients recruited from certain geographic regions had significantly worse adherence to treatment (when measured biochemically), which may have made the overall result a 'false negative' [48].

NEPRILYSIN INHIBITION

Neprilysin [also known as neutral endopeptidase (NEP)] degrades natriuretic and other vasoactive peptides (including bradykinin, substance P, endothelin and angiotensin II) and therefore neprilysin inhibition (NEPi) enhances the activity of the natriuretic peptide system leading to natriuresis, diuresis, BP reduction and inhibition of RAS and the sympathetic nervous system [49]. Isolated NEPi causes reflex activation of the RAS, so development of NEPi has always been combined with ACEi or ARB. The potential of NEPi in HFrEF was suggested in the Omapatrilat versus Enalapril Randomized Trial of Utility in Reducing Events trial, which compared omapatrilat (a combined ACEi and NEPi) to enalapril in 5770 patients with HF and found a non-significant 6% (95% CI −3–14) reduction in the primary outcome of all-cause mortality or hospitalization for HF [50]. However, development of omapatrilat was stopped when the Omapatrilat Cardiovascular Treatment Assessment Versus Enalapril trial (in 25 302 patients with hypertension) found an excess risk of angioedema compared with enalapril (2.17 versus 0.68%; $P < 0.005$) [51]. This was thought to be due to excessive bradykinin concentrations (as both ACE and NEP degrade bradykinin) and led to the development of a new class of drug called an angiotensin receptor neprilysin inhibitor (ARNI), which combines NEPi with an ARB.

Sacubitril/valsartan is a first-in-class ARNI that is rapidly metabolized after ingestion to the NEPi pro-drug sacubitril and the ARB valsartan. Sacubitril/valsartan reduces BP more than equivalent doses of valsartan alone [52]. The Prospective Comparison of ARNI with ACEi to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) trial randomized 8442 participants with HFrEF to treatment with sacubitril/valsartan or enalapril and was terminated earlier than planned based on the recommendation by the Data Monitoring Committee after interim efficacy analysis showed overwhelming evidence of benefit at a median follow-up duration of 27 months. Compared with those assigned to enalapril, participants assigned to sacubitril/valsartan in PARADIGM-HF experienced a 20% (95% CI 13–27) reduction in the primary composite endpoint of cardiovascular death or HF hospitalization. This effect was again similar among participants with and without CKD. Sacubitril/valsartan is now recommended in the European Society of Cardiology guidelines as a replacement for ACEi (or ARB) in patients who have symptomatic HF with a reduced LVEF $\leq$35% and who remain symptomatic despite maximum-tolerated evidence-based treatment [29, 40].

Sacubitril/valsartan has also been tested among patients with HFpEF. The PARAMOUNT trial compared sacubitril/valsartan with valsartan in 301 patients with change in NT-proBNP as the primary outcome [53]. At 12 weeks, among participants assigned sacubitril/valsartan, NT-proBNP was 23% (95% CI 8–36) lower compared with participants assigned valsartan. The PARAGON-HF trial has recruited 4822 participants with HFpEF to compare sacubitril/valsartan with valsartan and is scheduled to be completed in mid-2019 [54]. The primary outcome is the composite of cardiovascular death and total (first and recurrent) hospitalizations for HF.

In addition to its known benefits in HFrEF (and potential for benefit in HFpEF), NEPi might also have beneficial effects on the kidney. Experiments using 5/6 nephrectomy models suggested that NEPi reduces proteinuria and histological markers of kidney damage more than ACE inhibition alone [55, 56]. In addition, sacubitril/valsartan appeared to slow the deterioration of kidney function in the PARADIGM-HF [57] and PARAMOUNT trials [58]. However, it also modestly increased albuminuria in both trials (although baseline levels were very low in these HF populations) [59].

The UK Heart and Renal Protection (HARP)-III trial was designed to investigate the short- to medium-term effects of sacubitril/valsartan 97/103 mg twice daily versus irbesartan 300 mg once daily on kidney function among patients with established CKD [60]. Patients were eligible for the UK HARP-III trial if either their eGFR was $\geq$20–<45 mL/min/1.73 m^2 or their eGFR was $\geq$45–<60 mL/min/1.73 m^2 and the urine albumin:creatinine ratio was >20 mg/mmol. Other pre-specified outcomes included albuminuria, BP and cardiac biomarkers. A total of 414 participants were randomized and the average eGFR was 35 mL/min/1.73 m^2 and median urine albumin:creatinine ratio was 54 mg/mmol. Only 4 and 13% reported HF and coronary heart disease, respectively, at baseline.

The primary outcome of measured GFR at 12 months did not differ between the two groups: the difference in means was −0.1 (standard error 0.7) mL/min/1.73 m^2 [61]. Albuminuria was not significantly reduced [9% (95% CI −1–18) among those assigned sacubitril/valsartan] despite an additional 5.4/2.1 (both $P < 0.001$) mmHg reduction in BP. Despite the apparent lack of an effect on short to medium-term kidney function, allocation to sacubitril/valsartan did reduce both NT-proBNP and troponin I compared with allocation to irbesartan. Study average concentations of NT-proBNP and troponin I were 18% (95% CI 11–25) and 16% (95% CI 8–23) lower, respectively.

Although the effects on kidney function are not encouraging, they do not exclude a benefit on long-term progression of CKD (although any effect would not be large). However, the effects on BP and cardiac biomarkers support the hypothesis that sacubitril/valsartan might reduce the risk of cardiovascular events (and in particular those related to HF) among patients with CKD, irrespective of whether they have known cardiac disease. The neutral effects on tolerability and safety outcomes in the UK HARP-III trial would also support further investigation of this hypothesis.

CONCLUSION

The burden of HF among patients with CKD is considerable and contributes significantly to the excess of cardiovascular morbidity and mortality observed in this growing population. The anatomical substrates of HF develop early in the progression of CKD and strategies to prevent it have not been rigorously tested in the CKD population. Furthermore, trials among patients with known HF have usually excluded patients with moderate or advanced CKD, so the efficacy and—importantly—the safety of these treatments in the CKD population are uncertain. NEPi looks promising as a treatment that could reduce the risk of HF safely among patients with CKD, but clinical outcome trials are required. Newer treatments for HF, such as sodium glucose co-transporter-2 inhibitors, are being tested in large trials in both HF and CKD populations [62–64] and may be the first treatments that have proven efficacy for HF among patients with a wide-spectrum of kidney disease. Nevertheless, further trials of established and future interventions are required that allow doctors to confidently reduce excess risk of cardiovascular disease in CKD.

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CONFLICT OF INTEREST STATEMENT

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Appendix 13:

Publication: observational epidemiology of blood pressure and vascular outcomes from the SHARP trial:

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OPEN

Evidence for Reverse Causality in the Association Between Blood Pressure and Cardiovascular Risk in Patients With Chronic Kidney Disease

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Abstract—Among those with moderate-to-advanced chronic kidney disease, the relationship between blood pressure (BP) and cardiovascular disease seems U shaped but is loglinear in apparently healthy adults. The SHARP (Study of Heart and Renal Protection) randomized 9270 patients with chronic kidney disease to ezetimibe/simvastatin versus matching placebo and measured BP at each follow-up visit. Cox regression was used to assess the association between BP and risk of cardiovascular disease among (1) those with a self-reported history of cardiovascular disease and (2) those with no such history and, based on plasma troponin-I concentration, a low probability of subclinical cardiac disease. A total of 8666 participants had a valid baseline BP and troponin-I measurement, and 2188 had at least 1 cardiovascular event during follow-up. After adjustment for relevant confounders, the association between systolic BP and cardiovascular events was U shaped, but among participants without evidence of previous cardiovascular disease, there was a positive loglinear association throughout the range of values studied. Among those with the lowest probability of subclinical cardiac disease, each 10 mm Hg higher systolic BP corresponded to a 27% increased risk of cardiovascular disease (hazard ratio, 1.27; 95% confidence interval, 1.11–1.44). In contrast, the relationship between diastolic BP and cardiovascular risk remained U shaped irrespective of cardiovascular disease history or risk of subclinical disease. In conclusion, the lack of a clear association between systolic BP and cardiovascular risk in this population seems attributable to confounding, suggesting that more intensive systolic BP reduction may be beneficial in such patients.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00125593.

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Key Words: blood pressure ■ chronic kidney disease ■ epidemiology ■ vascular disease ■ troponin

In apparently healthy adults, each 20 mm Hg increase in long-term average—usual—systolic blood pressure (SBP) or 10 mm Hg higher usual diastolic blood pressure (DBP) is associated with about a doubling in the risk of death from ischemic heart disease, stroke, or heart failure, with no threshold level below which lower SBP is not associated with lower risk (at least down to 115/75 mm Hg).[1] Meta-analyses of randomized trials have demonstrated that lowering SBP reduces cardiovascular risk, confirming that the relationship between blood pressure (BP) and cardiovascular risk is one of the cause and effect.[2,3]

Chronic kidney disease (CKD) is a cause of hypertension and is associated with a high risk of cardiovascular disease.[4] Most patients with CKD die before reaching end-stage renal disease, and cardiovascular disease is the single largest cause of death among such patients.[4] However, in contrast to studies in apparently healthy people, observational studies of people with CKD have not consistently yielded a positive association between BP and cardiovascular risk, and at low–normal BP, some studies have indicated an increased risk of cardiovascular disease.[5–10] It has been suggested that this observation may be attributable to reverse causality, whereby long-standing

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hypertension causes changes in cardiac structure and function which lower BP while also increasing cardiovascular risk.[11,12]

If such a mechanism is indeed responsible, then it may be hypothesized that a positive association between BP and cardiovascular disease might be present among selected patients with CKD but without cardiac disease. Among patients with advanced CKD (ie, stages 4–5), at least 50% have echocardiographic evidence of abnormal cardiac structure,[13,14] many without any obvious clinical manifestations.[15] A potential surrogate measure of subclinical cardiac disease is provided by plasma troponin concentration, which correlates positively with left ventricular mass,[16,17] correlates negatively with cardiac function,[18] and predicts development of heart failure in unselected populations[19,20] and in people with CKD.[21] We hypothesized that there would be a trend toward a more strongly positive association between BP and cardiovascular events among those with the lowest baseline troponin-I concentrations (and hence the lowest risk of subclinical cardiac disease) in SHARP (Study of Heart and Renal Protection), a randomized trial comparing the combination of ezetimibe plus simvastatin versus placebo among 9270 patients with CKD.[22]

Methods

The trial methods and results have been published previously.[22] Patients aged 40 years or over were eligible to participate if they had at least 2 previous measurements of serum or plasma creatinine ≥150 µmol/L (≥1.7 mg/dL) in men or ≥130 µmol/L (≥1.5 mg/dL) in women or were receiving maintenance dialysis. Individuals with a previous history of myocardial infarction or coronary revascularization were excluded, but individuals with a history of angina, peripheral vascular disease, stroke, or diabetes mellitus were eligible. In the current analyses, baseline information refers to information that was recorded at randomization to ezetimibe/simvastatin versus placebo (or shortly before). Baseline information included sociodemographic characteristics (age, sex, ethnicity, and highest attained educational achievement), anthropometric measurements, self-reported medical history, current medication (including antihypertensive treatments, but not their doses), and lifestyle behaviors (alcohol consumption and smoking).

At each study clinic visit, using a suitably sized cuff attached to an automated digital sphygmomanometer (UA-767; A&D Company, Ltd, Tokyo, Japan), trained research nurses recorded a single BP reading after the patient had been seated for 5 minutes.

Baseline samples of nonfasting blood and urine were collected and stored at or below −40°C before transfer to the accredited central laboratory. Creatinine was measured using a kinetic alkaline picrate method calibrated using material traceable to National Institute of Standards and Technology Standard Reference Material 914a, and estimated glomerular filtration rate (eGFR) calculated using the CKD-EPI study (CKD Epidemiology Collaboration) equation.[23] Troponin-I was measured by chemiluminescent immunoassay on an ACCESS2 analyzer using AccuTnI reagent and calibrator (Beckman Coulter Inc) and Liquichek Cardiac Markers Plus Controls (Bio-Rad Laboratories Ltd). Assay linearity and functional sensitivity was verified down to at least 0.01 ng/mL.

After randomization, participants were followed up at 2 and 6 months and then at 6 monthly intervals for at least 4 years. Wherever possible, follow-up of patients who were unable to attend clinics was conducted by telephone. At each follow-up, information on all serious adverse events (including all hospitalizations) was sought, and further supporting documentation collected on events that might have represented a study outcome. These documents were sent for central adjudication by trained clinicians blind to randomized treatment allocation using prespecified criteria. For the purpose of the present analyses, we defined the following outcomes (1) atherosclerotic cardiovascular event (myocardial infarction, coronary death, unstable angina, ischemic heart failure, coronary revascularization, nonhemorrhagic stroke, transient ischemic attack, and peripheral arterial disease diagnosis, including noncoronary revascularization), (2) nonatherosclerotic cardiovascular event (other cardiac death, nonischemic heart failure, arrhythmia, valvular heart disease, and hemorrhagic stroke), and (3) any cardiovascular event (atherosclerotic and nonatherosclerotic cardiovascular events combined). Analyses of nonvascular mortality were included for comparison.

Statistical Analysis

The relationship between baseline troponin-I (≤0.01 ng/mL; >0.01 but ≤0.03 ng/mL; and >0.03 ng/mL) and risk of cardiovascular events in the SHARP trial was assessed in Cox models adjusting for age, sex, ethnicity (white, black, Asian, and other), country, highest attained educational achievement (university, secondary school, vocational qualification, other, and unrecorded), smoking (never, former, and current), self-reported diabetes mellitus, body mass index, renal replacement therapy status (dialysis or not), eGFR, BP, and randomized treatment allocation.

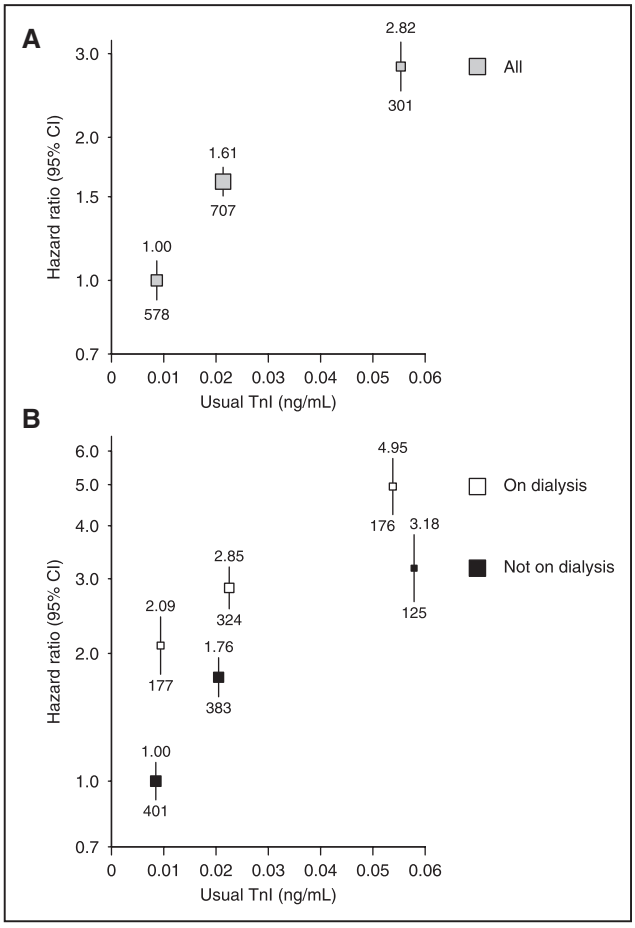

Figure 1. Association between troponin-I (TnI) and risk of cardiovascular events (**A**) overall and (**B**) by renal replacement therapy status. Analyses restricted to those without previous cardiovascular disease at baseline. The reference group in **A** is those with a TnI ≤0.01 ng/mL and in **B**, it is those not on dialysis at baseline with a TnI ≤0.01 ng/mL. Hazard ratios adjusted for age, sex, ethnicity, country, education, smoking status, previous diabetes mellitus, estimated glomerular filtration rate, renal replacement therapy status (**A** only), body mass index, treatment allocation, and blood pressure are quoted (above squares) with number of events (below squares). CI indicates confidence interval.

Table. Baseline Characteristics and Measurements by Thirds of Baseline Blood Pressure

Characteristic/Measurement	SBP				DBP			
	Bottom Third, n=3123	Middle Third, n=3015	Top Third, n=3119	*P* Value*	Bottom Third, n=3084	Middle Third, n=3143	Top Third, n=3019	*P* Value†
Blood pressure								
Baseline systolic, mm Hg	116 (10)	138 (5)	163 (14)	<0.0001	127 (20)	138 (18)	152 (20)	<0.0001
Baseline diastolic, mm Hg	72 (10)	80 (10)	86 (12)	<0.0001	65 (6)	79 (3)	93 (7)	<0.0001
Usual systolic, mm Hg	128 (4)	136 (2)	143 (4)	<0.0001	132 (7)	136 (6)	140 (6)	<0.0001
Usual diastolic, mm Hg	74 (4)	77 (4)	80 (5)	<0.0001	71 (3)	77 (1)	82 (2)	<0.0001
Any antihypertensive medication (%)‡	81	85	87	<0.0001	83	84	86	0.0038
Demographics								
Age at randomization, y	60 (12)	62 (12)	63 (12)	<0.0001	66 (12)	62 (11)	58 (11)	<0.0001
Men (%)	57	63	68	<0.0001	59	62	67	<0.0001
Previous disease								
Evidence of previous cardiovascular disease, including Troponin-I >0.01 (%)‡	49	51	59	<0.0001	56	52	52	0.0031
Self-reported history of cardiovascular disease (%)	15	16	17	0.03	18	15	14	0.0002
Troponin-I, ng/mL (%)				<0.0001				0.05
≤0.01	58	56	47		52	55	54	
>0.01, ≤0.03	33	34	39		37	35	35	
>0.03, ≤0.1	7	8	11		9	9	9	
>0.1	2	2	2		2	2	1	
Diabetes mellitus (%)‡	18	22	28	<0.0001	28	22	17	<0.0001
Renal replacement therapy status (%)‡								
Not on dialysis	66	70	66	0.0008	59	70	72	<0.0001
On dialysis	34	30	34	0.0007	41	30	27	<0.0001
Renal function								
CKD-EPI–estimated GFR, mL/min/1.73m^2‡§								
Mean (SD)	26.2 (12.8)	25.3 (12.7)	24.5 (12.8)	0.0001	25.1 (13.0)	25.5 (12.7)	25.4 (12.9)	0.60
≥60 (%)	2	2	<1		1	2	1	
≥30, <60 (%)	33	30	31		30	32	31	
≥15, <30 (%)	46	44	43		45	43	45	
<15 (%)	20	24	26		23	23	23	
Urinary albumin:creatinine ratio, mg/g§								
Geometric mean (approximate SE)	94 (4)	173 (7)	302 (13)	<0.0001	118 (5)	171 (7)	240 (10)	<0.0001
<30 (%)	27	19	12		26	18	15	
≥30, ≤300 (%)	44	38	32		38	42	34	
>300 (%)	28	42	55		35	40	51	

Mean (SD); % or, geometric mean (SE) are shown. There were 9270 participants randomized, but 604 had missing values of SBP, DBP, or previous cardiovascular disease at baseline and are excluded from all analyses. Among the 5854 included participants not on dialysis at baseline, 32 (0.5%) and 471 (8%) had missing values for baseline estimated GFR and urine albumin:creatinine ratio, respectively. CKD-EPI indicates chronic kidney disease Epidemiology Collaboration; DBP, diastolic blood pressure; GFR, glomerular filtration rate; and SBP, systolic blood pressure.

*Test of heterogeneity between SBP categories.

†Test of heterogeneity between DBP categories.

‡Adjusted for age, sex, and ethnicity.

§For participants not on dialysis.

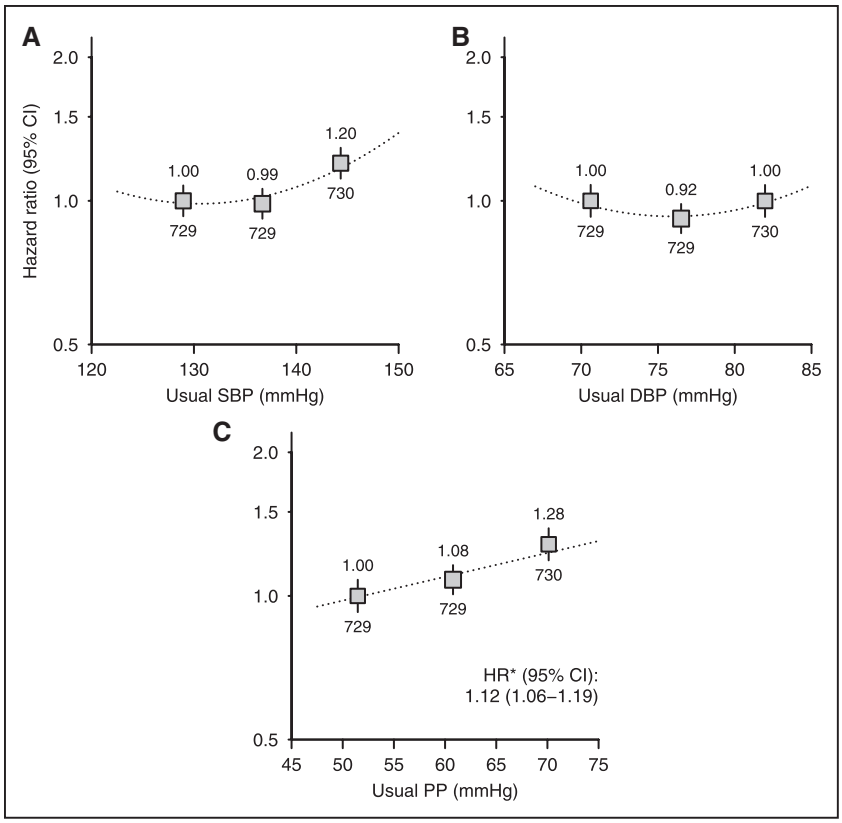

Figure 2. Association between (**A**) systolic blood pressure (SBP), (**B**) diastolic blood pressure (DBP), and (**C**) pulse pressure (PP) and cardiovascular events overall. For each plot, categories of blood pressure contain similar numbers of events. Hazard ratios (HRs) adjusted for age, sex, ethnicity, country, education, smoking status, previous cardiovascular disease, previous diabetes mellitus, estimated glomerular filtration rate, renal replacement therapy status, body mass index, and treatment allocation are quoted (above squares) with numbers of events (below). Exclusions as per Table.*HRs per 10 mm Hg higher usual blood pressure are presented for associations where there is no evidence of deviation from a loglinear relationship. CI indicates confidence interval.

Assumptions about the nature and direction of any causal or effect modifying relationships between baseline characteristics, BP, and outcomes were formulated a priori (see directed acyclic graph in Figure S1 in the online-only Data Supplement).[24] SBP, DBP, and their difference (pulse pressure [PP]) as continuous variables were related to the risk of cardiovascular events using Cox proportional hazards regression adjusted for previous cardiovascular disease and the same variables used in the troponin model above. Because our a priori assumption was that urinary albumin excretion is a mediating variable (ie, BP influences risk partly through its effects on urinary albumin excretion; Figure S1), we did not adjust for this variable in our primary model, although we did so in exploratory analyses. To adjust for variation in BP, we applied a standard correction for regression dilution bias.[25] Such adjustment allows the relevance of long-term average—usual—BP to be quantified but does not affect the statistical assessment of nonlinearity (Methods in the online-only Data Supplement; Figure S2).[26] To test for nonlinear associations, models for the main analyses were additionally fitted with a quadratic BP function. A quadratic function was retained if the difference in twice the log-likelihood statistic between 2 nested models (one with and the other without the quadratic function) provided statistical evidence for improvement in model fit (ie, there was evidence of a nonlinear association), and the *P* value for this comparison referred to as the test for nonlinearity. Heterogeneity testing was performed to assess whether associations differed between participants by the selected subgroups (reported cardiovascular disease versus none; and among those with no such report, by troponin-I ≤0.01 versus >0.01 ng/mL) using an analogous method, including where relevant an additional interaction term between evidence of previous cardiovascular disease and a quadratic function of BP.

In figures displaying associations between BP and risk, for each subgroup, hazard ratios (HRs) were presented for 3 groups containing an equal numbers of events with regression lines calculated from regression models using BP as a continuous variable, and these plotted against the mean BP value at the study midpoint accompanied by a confidence interval (CI) derived only from the variance of the log risk in that 1 group. Hence, each HR, including that for the reference group, was associated with a group-specific CI that reflects the amount of data only in that 1 group, thereby allowing appropriate statistical comparisons to be made between any 2 groups.[27]

Values for the small number of missing eGFR and urinary albumin:creatinine ratio were imputed using multiple imputation, with the results across imputations combined using the methods of Rubin.[28] In sensitivity analyses, the main analyses were repeated separately among participants on dialysis and those not, and among those above and below the study's median age. The proportional hazard assumption was tested through examination of the time dependency of the Schoenfeld partial residuals. Analyses used SAS v9.3 (SAS Institute, Cary, NY) and R v2.14.2.

Results

A total of 604 participants were excluded from analyses due either to a missing baseline measurement of BP (n=25 individuals) or a missing troponin-I measurement (n=579). Of the remaining 8666 participants, 7278 reported no previous history of cardiovascular disease, and among this group, a higher baseline troponin-I was associated with male sex, higher SBP, older age, more diabetes mellitus, and worse renal function (with a larger proportion of such patients on dialysis; Table S1). After adjustment for these differences, increasing baseline troponin-I was strongly associated with future cardiovascular risk. Compared with those with a troponin-I ≤0.01 ng/mL, those with troponin-I concentration >0.01 but ≤0.03 ng/mL, and >0.03 ng/mL were at 61% (HR, 1.61; 95% CI, 1.43–1.81) and 182% (HR, 2.82; 95% CI, 2.42–3.28) increased cardiovascular risk, respectively (Figure 1A). A higher troponin-I was associated with increased cardiovascular risk in both dialysis and nondialysis patients (Figure 1B).

Mean baseline SBP ranged from 116 mm Hg in the lowest third to 163 mm Hg in the highest third. Compared with those

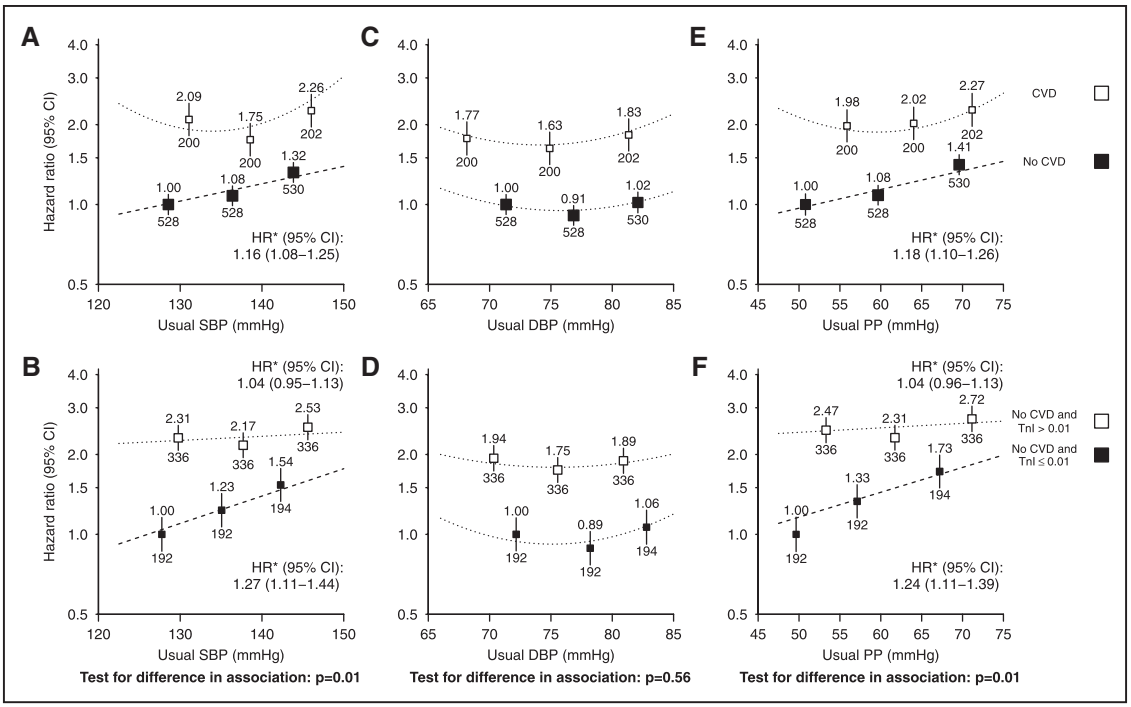

Figure 3. Association between systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP) and cardiovascular events, subdivided by self-reported history of previous cardiovascular disease (**A**, **C**, **E**) and by baseline troponin-I concentration (**B**, **D**, **F**). For each plot, categories of blood pressure contain similar numbers of events. Hazard ratios adjusted for age, sex, ethnicity, country, education, smoking status, previous diabetes mellitus, estimated glomerular filtration rate, renal replacement therapy status, body mass index, and treatment allocation are quoted (above squares) with numbers of events (below). Exclusions as per Table. *Hazard ratios per 10 mm Hg higher usual SBP/PP are presented for associations where there is no evidence of deviation from a log–linear relationship. CI indicates confidence interval; CVD, self-reported history of cardiovascular disease; HR, hazard ratio; and TnI, troponin-I (ng/mL).

in the lowest third, those in the highest third of SBP were more often male, were older, and reported more diabetes mellitus and previous cardiovascular disease, and nondialysis patients had lower eGFR (Table; Table S2). Mean baseline DBP ranged from 65 mm Hg in the lowest third to 93 mm Hg in the highest third. Compared with those in the lowest third of DBP, and in contrast to the baseline characteristics by SBP, those with higher DBP were younger, less likely to report diabetes mellitus, previous cardiovascular disease, or to be on dialysis (Table; Table S2). The majority of participants were taking at least 1 antihypertensive agent, ranging from 87% in the highest third of baseline SBP to 81% in the lowest third, and from 86% to 83% in the highest and lowest thirds of DBP, respectively (Table). Over one half of participants were taking at least 2 agents (Table S2).

Overall, 2188 participants experienced at least 1 cardiovascular event during a median of 4.9 years of follow-up (annual rate 6.7% per year).

SBP and Vascular Risk

The adjusted association between SBP and cardiovascular risk was U shaped (Figure 2A; test against the linearity assumption [nonlinearity] P=0.003). But, among the 7278 participants who reported no previous history of cardiovascular disease, there was a positive loglinear association throughout the range studied (Figure 3A; nonlinearity P=0.35). After adjusting for regression dilution, each 10 mm Hg higher usual SBP was associated with 16% higher cardiovascular risk (HR, 1.16; 95% CI, 1.08–1.25). Among this group, there was a steeper association in those with lower baseline troponin (heterogeneity test P=0.01; Figure 3B). Among those at lowest probability of cardiac disease (no self-reported previous cardiovascular disease and troponin-I ≤0.01 ng/mL), each 10 mm Hg higher usual SBP was associated with 27% higher cardiovascular risk (HR, 1.27; 95% CI, 1.11–1.44; Figure 3B). Additional adjustment for baseline urinary albumin:creatinine ratio had little impact on this estimated HR (1.23; 95% CI, 1.08–1.40).

The magnitude of association between SBP and risk of cardiovascular events was similar for atherosclerotic (HR per 10 mm Hg usual SBP, 1.25; 95% CI, 1.06–1.48) and nonatherosclerotic events (HR per 10 mm Hg usual SBP, 1.31; 95% CI, 1.09–1.57; Figure 4A and 4B). Within the low cardiac risk group, there were apparently similar loglinear associations between SBP and risk of cardiovascular events among those on dialysis and those not (HRs per 10 mm Hg higher SBP 1.36; 95% CI, 1.16–1.60 and 1.18; 95% CI, 0.95–1.47; heterogeneity P=0.31; Figure 5A and 5B), although these analyses were constrained by the small numbers of events. Likewise, there were apparently similar loglinear associations in those younger than 62 and those aged 62 years or over (HRs, 1.37; 95% CI, 1.14–1.66; 1.20; 95% CI, 1.00–1.43; heterogeneity P=0.31; Figure S3A and S3B).

DBP and Vascular Risk

Overall, there was a U-shaped association between DBP and cardiovascular events (nonlinearity P=0.0008; Figure 2B). This association was U shaped irrespective of a recorded history of cardiovascular disease or the probability of cardiac

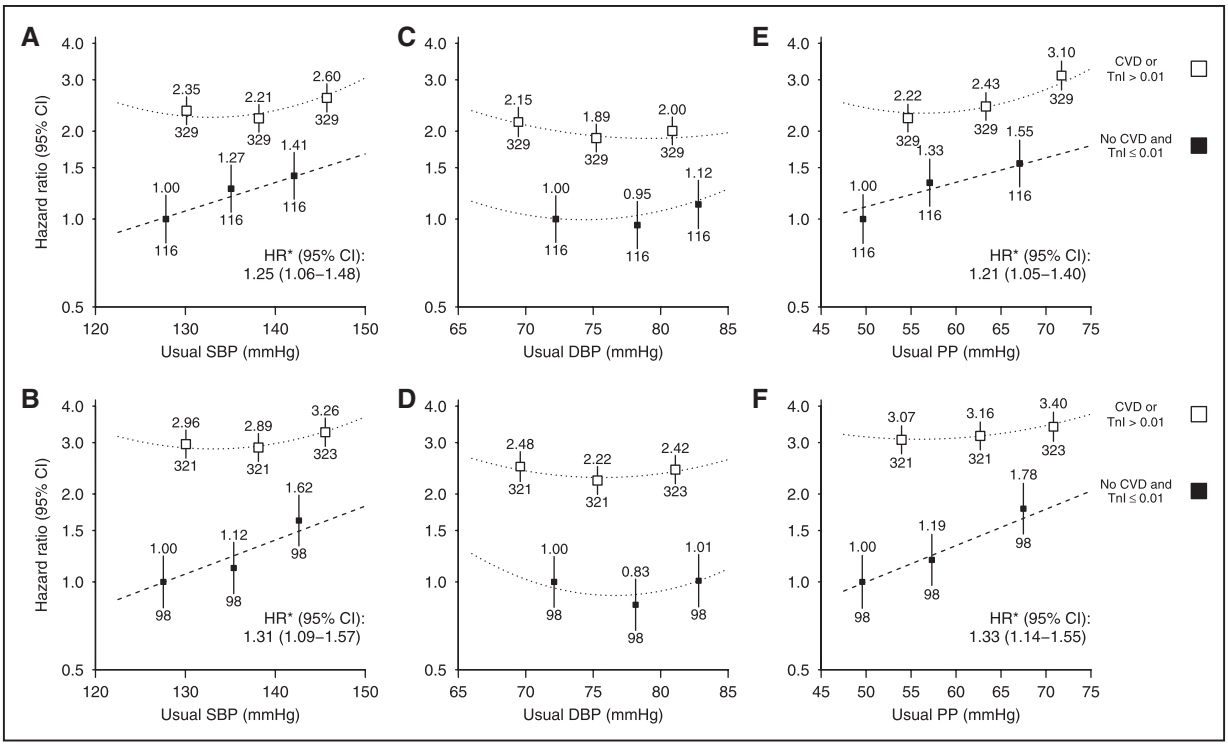

Figure 4. Association between (**A**) systolic blood pressure (SBP), (**C**) diastolic blood pressure (DBP), and (**E**) pulse pressure (PP) and atherosclerotic cardiovascular events and association between (**B**) SBP, (**D**) DBP, and (**F**) PP and nonatherosclerotic cardiovascular events, subdivided by evidence of previous cardiovascular disease. Conventions as per Figure 3. CI indicates confidence interval; CVD, self-reported history of cardiovascular disease; HR, hazard ratio; and TnI, troponin-I (ng/mL).

disease in those without such a history (Figure 3C and 3D) and was similar for both atherosclerotic and nonatherosclerotic events (Figure 4C and 4D), in dialysis and nondialysis (Figure 5C and 5D), and in younger and older patients (Figure S3C and S3D).

PP and Vascular Risk

Overall, the adjusted association between PP and risk of cardiovascular events was loglinear (HR per 10 mm Hg higher usual PP, 1.12; 95% CI, 1.06–1.19; Figure 2C) but was U shaped among those with a history of cardiovascular disease and loglinear among those without such a history (HR per 10 mm Hg higher usual PP, 1.18; 95% CI, 1.10–1.26; Figure 3E). Among those in the lowest category of troponin-I, each 10 mm Hg higher usual PP was associated with 24% higher cardiovascular risk (HR, 1.24; 95% CI, 1.11–1.39; Figure 3F), with similar relationships for atherosclerotic and nonatherosclerotic cardiovascular events considered separately (HRs per 10 mm Hg higher usual PP 1.21; 95% CI, 1.05–1.40 and 1.33; 95% CI, 1.14–1.55, respectively; Figure 4E and 4F). Among those at lowest cardiac risk, the HRs per 10 mm Hg higher PP were similar among dialysis and nondialysis (Figure 5E and 5F) and in younger and older patients (Figure S3E and S3F).

BP and Nonvascular Mortality

There were 1196 nonvascular deaths during follow-up (3.2% per year). For SBP, there was some evidence for a U-shaped association (nonlinearity P=0.03) with nonvascular mortality, while the relationship with DBP appeared flat (nonlinearity P=0.24; HR per 5 mm Hg usual DBP, 1.00; 95% CI, 0.94–1.06) and was similar irrespective of baseline troponin-I (Figure S4).

Discussion

A U-shaped association between BP and cardiovascular risk has been observed in many studies of populations with advanced CKD,[5–10] which is in contrast to the positive loglinear relationships with ischemic heart disease, stroke, and heart failure mortality observed among apparently healthy adults.[1] The presence of a clear positive loglinear relationship between SBP (or PP) and cardiovascular events in patients with CKD at lowest risk of cardiac disease in SHARP suggests that reverse causality is a plausible explanation for previously observed U-shaped associations among patients with moderate-to-advanced CKD.[5–10] A loglinear relationship between SBP (or PP) and the risk of cardiovascular events was present in both dialysis and nondialysis patients, suggesting that BP remains a cause of cardiovascular disease irrespective of the severity of CKD, and hence that the absolute benefits of lowering BP among dialysis patients may be larger than those achievable at an earlier stage of CKD.

We did not observe a positive association between DBP and cardiovascular risk in this population. Myocardial perfusion is dependent on diastolic blood flow, and it has been suggested that a hypertrophied left ventricle (a key feature of structural heart disease in CKD[13,14]) may be more likely to become ischemic at low levels of DBP than a normal ventricle.[29] Because PP is the difference between SBP and DBP, our finding of a positive association between PP and cardiovascular risk in those at lowest risk of cardiac disease reflects

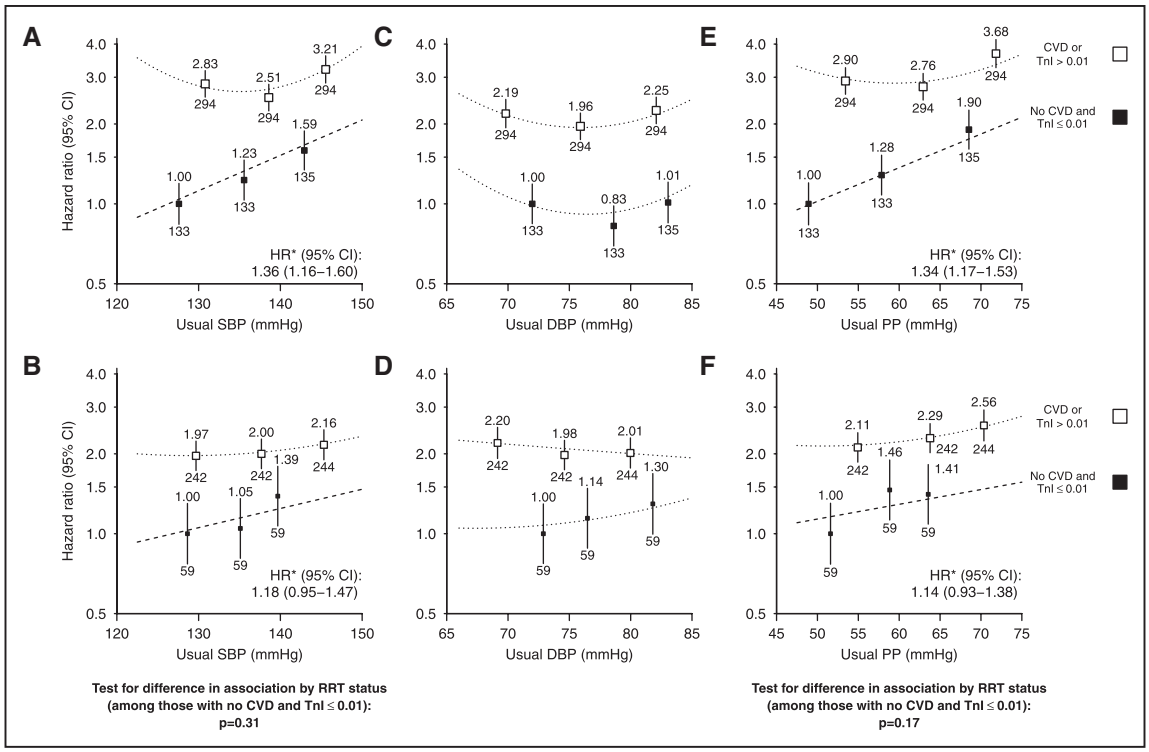

Figure 5. Association between systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP) and cardiovascular events, subdivided by evidence of previous cardiovascular disease, for those not on dialysis (**A**, **C**, **E**) and on dialysis (**B**, **D**, **F**). Conventions as per Figure 3. CI indicates confidence interval; CVD, self-reported history of cardiovascular disease; HR, hazard ratio; and TnI, troponin-I (ng/mL).

the finding of a positive relationship for SBP and a U-shaped relationship for DBP. Vascular calcification is accelerated in CKD and reduces vascular recoil, thereby increasing SBP and decreasing DBP, that is, widening PP.[30] If present, vascular calcification may increase the risk of cardiovascular events,[31] and the present analyses suggest that widening PP is associated with an increased risk of both atherosclerotic and nonatherosclerotic cardiovascular events in this population.

Among people with cardiovascular disease, randomized trials have shown that lowering BP is effective at reducing cardiovascular risk,[32] in spite of U-shaped associations between BP and cardiovascular risk being commonly observed in such populations.[29,33–35] Similarly, lowering BP is effective in elderly people,[36,37] in whom some prospective studies have also failed to demonstrate a positive association between BP and cardiovascular disease.[29] Comparatively few people with moderate-to-advanced CKD have been studied in trials of antihypertensive therapy, but about 10 000 people with some evidence of reduced renal function were included in a recent meta-analysis.[3] In this study, each 5 mm Hg SBP reduction lowered cardiovascular risk by 14%, with no heterogeneity in this risk reduction among different categories of eGFR.[3] Similar benefits were observed in a separate meta-analysis of trials conducted among people on dialysis.[38] However, although BP lowering seems beneficial in CKD, the optimum BP target for people with CKD is unknown, with current guideline recommendations ranging from <130/80 to <150/90 mm Hg (Table S3).

There have been 2 negative trials of intensive versus standard BP lowering in CKD populations, but these lacked statistical power to detect the magnitude of benefit suggested by our analyses.[39,40] The recent SPRINT (Systolic Blood Pressure Intervention Trial) demonstrated clearly that an SBP target of 120 mm Hg (achieved SBP 121 mm Hg) was superior to a target of 140 mm Hg (achieved SBP 136 mm Hg) in high-risk adults.[37] These data, taken together with the evidence of reverse causality in the present analysis in the SHARP trial, suggest that trials of lower BP targets in patients with CKD are indicated. Such trials would also be able to assess the potential hazards of lower BP targets—for example, in SPRINT, the more intensive BP regimen was associated with an excess of acute kidney injury (204/4678 [4.4%] versus 120/4683 [2.6%]; $P<0.001$)[37]—and the somewhat uncertain benefits of intensive BP lowering on renal progression.

Our study has the advantage of a large sample size, detailed adjudication of cardiovascular events, and the ability to select those at lowest risk of cardiac disease through the measurement of baseline troponin (which has not been possible in previous studies[5–10]). The most important limitation is that, because no cardiac imaging was performed in SHARP, the correlation between troponin-I concentration and preexisting structural cardiac disease cannot be formally confirmed in this cohort. Nevertheless, the use of troponin as a tool to identify those at higher risk of subclinical cardiac disease is supported by other studies,[16–21,41] and baseline troponin-I was a strong independent predictor of cardiovascular risk in both dialysis and nondialysis patients in SHARP. A further limitation is that SHARP only had a single measurement of BP at each clinic visit, which means short-term variability in BP was not assessed. This may also lead to underestimates of the strength of the relationship between BP and cardiovascular

risk, particularly because BP exhibits marked day-to-day variability among people on dialysis in whom out-of-dialysis unit SBP readings give better estimates of average BP than measurements taken before or after dialysis.[42,43] This limitation was partially offset by our adjustment for regression dilution bias. Such adjustment is well established in studies of apparently healthy individuals[25] because the magnitude of reductions in cardiovascular risk produced by antihypertensive therapy in randomized trials[2,3] is better predicted by associations between usual, rather than a single measure of BP in observational studies.[1–3]

Perspectives

In summary, a U-shaped association between SBP and cardiovascular risk in CKD populations, as observed in many previous studies, may be attributable to reverse causality because of subclinical cardiac disease. When adjustment is made for such confounding, the observed association between SBP and both atherosclerotic and nonatherosclerotic cardiovascular risk is positive and loglinear, consistent with BP being a causal risk factor for both forms of cardiovascular disease in patients with CKD, as it is in other populations. Randomized trials of more intensive BP reduction should be a priority in patients with moderate-to-advanced CKD.

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Novelty and Significance

What Is New?

- Observational studies have found that the association between systolic blood pressure (SBP) and cardiovascular risk in chronic kidney disease (CKD) populations is U shaped. We have shown that the U-shaped relationship is confined to patients with a known history of cardiovascular disease or a high probability of such disease, whereas among patients with a lower probability of subclinical cardiovascular disease, there is a loglinear association between SBP (or pulse pressure) and both atherosclerotic and nonatherosclerotic cardiovascular diseases.

What Is Relevant?

- These observations indicate that confounding by disease is the chief explanation for the apparent weakening and reversal of the association between SBP and cardiovascular risk in moderate-to-advanced CKD and suggest that such confounding masks a causal association between blood pressure and risk in patients with CKD with established cardiovascular disease. They support the need for randomized trials of more versus less intensive blood pressure reduction among patients with moderate-to-advanced CKD, including hemodialysis and peritoneal dialysis patients.

Summary

This study examined the association between blood pressure and risk of cardiovascular disease among CKD patients with (1) no self-reported history of cardiovascular disease and (2) no such history and, based on plasma troponin-I concentration, a low probability of subclinical cardiac disease. Overall, the association between SBP and cardiovascular events was U shaped, but among participants without evidence of previous cardiovascular disease, there was a positive loglinear association with SBP throughout the range of values studied. Among those with the lowest probability of subclinical cardiac disease, each 10 mm Hg higher SBP corresponded to a 27% increased risk of cardiovascular disease.

Supplemental Material

Evidence for reverse causality in the association between blood pressure and cardiovascular risk in patients with chronic kidney disease

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Detailed Statistical Methods: Estimation of "Usual" Blood Pressure

To ensure natural blood pressure variation and any measurement error was accounted for, a standard correction for such regression-dilution bias was made. (Supplemental Figure 2).[1,2] Each individual's usual systolic blood pressure, S, was estimated using linear regression models with blood pressure at the study midpoint (2.5 years) as the outcome and their baseline value, s, as the explanatory variable. It was found that there was a quadratic relationship between baseline and follow-up blood pressure, so usual systolic blood pressure was estimated using the formula:

$$S=136.1 + 0.316(s - 138.9) - 0.001(s - 138.9)^2.$$

Similarly, each individual's usual diastolic blood pressure, D, was calculated from their baseline value, d, using the formula:

$$D=77.1 + 0.396(d - 79.1) - 0.0018(d - 79.1)^2.$$

A similar method of estimation of usual blood pressure has been used previously in the analyses of the influence of blood pressure on vascular disease risk performed by the Prospective Studies Collaboration.[3]

The following hazard ratios demonstrate how the use of a single blood pressure measurement or the average of 3 readings over 6 months would underestimate the relevance of SBP to vascular risk (among those who reported no previous history of cardiovascular disease and a baseline troponin-I ≤0.01ng/mL) compared to using the usual SBP described above.

	Hazard ratio (95% CI) per 10 mmHg higher SBP
"Usual" SBP	1.29 (1.12-1.48)*
Average SBP of 3 readings over 6 months	1.11 (1.05-1.16)
Single baseline measure of SBP	1.08 (1.04-1.13)

SBP = systolic blood pressure. *The hazard ratio quoted here for "usual" SBP differs to that quoted in Figure 3 as these analyses exclude participants with missing values of SBP at 2 or 6 months.

Statistical References

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Table S1: Baseline characteristics and laboratory measurements subdivided by self-reported history of prior cardiovascular disease and baseline troponin-I concentration

	Self-reported history of previous cardiovascular disease and baseline troponin-I concentration		
	No CVD		
Characteristic/measurement	**TnI≤0.01 (n=4070)**	**TnI>0.01 (n=3208)**	**CVD (n=1388)**
Blood pressure			
Baseline systolic (mmHg)	136 (20)	142 (23)	141 (23)
Baseline diastolic (mmHg)	80 (12)	79 (13)	76 (13)
Usual systolic (mmHg)	135 (6)	136 (7)	136 (7)
Usual diastolic (mmHg)	77 (5)	77 (5)	76 (5)
Any antihypertensive medication	3405 (84%)	2722 (85%)	1194 (86%)
Demographics			
Age at randomization (years)	59 (11)	64 (12)	67 (11)
Men	2338 (57%)	2193 (68%)	910 (66%)
Ethnicity			
White	2985 (73%)	2222 (69%)	1033 (74%)
Black	73 (2%)	104 (3%)	41 (3%)
Asian	908 (22%)	786 (25%)	272 (20%)
Other	104 (3%)	96 (3%)	42 (3%)
Education			
University	569 (14%)	307 (10%)	120 (9%)
Secondary school	1377 (34%)	1016 (32%)	435 (31%)
Vocational qualifications	891 (22%)	768 (24%)	366 (26%)
Primary school or no formal education	651 (16%)	647 (20%)	287 (21%)
Not specified	582 (14%)	470 (15%)	180 (13%)
Current smoker	560 (14%)	387 (12%)	207 (15%)
Prior disease			
Self-reported history of cardiovascular disease	0 (0%)	0 (0%)	1388 (100%)
Troponin-I (ng/mL)			
≤0.01	4070 (100%)	0 (0%)	527 (38%)
>0.01 to ≤0.03	0 (0%)	2502 (78%)	551 (40%)
>0.03 to ≤0.1	0 (0%)	591 (18%)	186 (13%)
>0.1	0 (0%)	115 (4%)	40 (3%)
Diabetes	621 (15%)	859 (27%)	506 (36%)
Renal status			
Not on dialysis	3187 (78%)	1731 (54%)	926 (67%)
On dialysis	878 (22%)	1474 (46%)	460 (33%)
Measurements			
CKD-EPI-estimated GFR (mL/min/1.73m²)*			
Mean (SD)	26.6 (13.3)	23.2 (12.0)	25.0 (13.0)
≥60	49 (1%)	20 (1%)	13 (1%)
≥30 to <60	1127 (28%)	426 (13%)	263 (19%)
≥15 to <30	1375 (34%)	790 (25%)	411 (30%)
<15	639 (16%)	498 (16%)	211 (15%)
Urinary albumin:creatinine ratio (mg/g)*			
Median (IQR)	175 (37-645)	253 (60-896)	224 (49-979)
<30	648 (16%)	267 (8%)	153 (11%)
≥30 to ≤300	1159 (28%)	596 (19%)	293 (21%)
>300	1152 (28%)	752 (23%)	363 (26%)
Body-mass index (kg/m²)	27.0 (5.3)	27.0 (5.7)	27.4 (5.6)
Treatment allocation			
Randomized to simvastatin plus ezetimibe	2014 (49%)	1630 (51%)	709 (51%)

Mean (SD) or n (%) shown. GFR=glomerular filtration rate. CVD = self-reported history of cardiovascular disease. TnI=troponin-I.
*For participants not on dialysis. Missing data as described in Table.

Table S2: Additional baseline characteristics and laboratory measurements by tertiles of baseline blood pressure

	Systolic blood pressure (SBP)				Diastolic blood pressure (DBP)			
Characteristic/measurement	**Bottom third (n=3123)**	**Middle third (n=3015)**	**Top third (n=3119)**	**P value***	**Bottom third (n=3084)**	**Middle third (n=3143)**	**Top third (n=3019)**	**P value†**
Other demographics								
Ethnicity				<0.0001				0.0016
White	74%	74%	67%		74%	72%	69%	
Black	3%	2%	3%		3%	3%	3%	
Asian	20%	21%	26%		21%	23%	24%	
Other	3%	3%	3%		3%	3%	4%	
Education				<0.0001				0.21
University	13%	12%	10%		11%	12%	12%	
Secondary school	32%	34%	31%		32%	34%	33%	
Vocational qualifications	22%	23%	24%		24%	22%	23%	
Primary school or no formal education	17%	17%	21%		19%	18%	19%	
Not specified	15%	14%	14%		15%	14%	14%	
Current smoker	12%	13%	14%	0.07	13%	12%	14%	0.03
Medications								
Number of antihypertensive medications				<0.0001				0.01
None	19%	15%	13%		17%	17%	14%	
One	26%	23%	23%		23%	25%	24%	
Two	24%	26%	25%		23%	25%	28%	
Three or more	30%	36%	38%		38%	33%	34%	
Type of antihypertensive medication								
ACE inhibitor or ARB	53%	55%	55%	0.12	54%	54%	55%	0.42
Beta blocker	36%	38%	39%	0.03	38%	36%	39%	0.03
Calcium channel blocker	32%	43%	48%	<0.0001	40%	41%	43%	0.09
Diuretic	41%	41%	42%	0.50	45%	40%	39%	<0.0001
Other co-medication								
Antiplatelet therapy	23%	22%	23%	0.88	27%	21%	19%	<0.0001
Oral anticoagulant therapy	4%	3%	3%	0.0021	4%	3%	3%	0.03
Erythropoiesis stimulating agent	26%	26%	29%	0.01	31%	26%	25%	<0.0001
Sevelamer	9%	7%	8%	0.17	10%	7%	7%	<0.0001

Table S2: Additional baseline characteristics and laboratory measurements by tertiles of baseline blood pressure

	Systolic blood pressure (SBP)				Diastolic blood pressure (DBP)			
Characteristic/measurement	**Bottom third (n=3123)**	**Middle third (n=3015)**	**Top third (n=3119)**	**P value***	**Bottom third (n=3084)**	**Middle third (n=3143)**	**Top third (n=3019)**	**P value†**
Other measurements								
Body-mass index (kg/m²)	26.8 (5.4)	27.0 (5.4)	27.4 (5.4)	0.0005	27.1 (5.5)	27.2 (5.4)	27.0 (5.5)	0.44
Total cholesterol (mmol/L)	4.83 (1.15)	4.91 (1.15)	4.91 (1.16)	0.01	4.75 (1.17)	4.89 (1.14)	5.02 (1.16)	<0.0001
LDL cholesterol (mmol/L)	2.74 (0.86)	2.80 (0.86)	2.78 (0.86)	0.02	2.68 (0.87)	2.78 (0.85)	2.86 (0.87)	<0.0001
HDL cholesterol (mmol/L)	1.11 (0.33)	1.12 (0.33)	1.13 (0.33)	0.09	1.08 (0.33)	1.12 (0.33)	1.15 (0.33)	<0.0001
Triglycerides (mmol/L)	2.31 (1.73)	2.34 (1.72)	2.32 (1.73)	0.81	2.38 (1.75)	2.28 (1.72)	2.31 (1.75)	0.07
Phosphate (mmol/L)	1.26 (0.44)	1.27 (0.44)	1.30 (0.44)	0.0008	1.30 (0.44)	1.26 (0.44)	1.27 (0.44)	0.0015
Hemoglobin (g/dL)	12.28 (1.66)	12.17 (1.65)	12.05 (1.64)	<0.0001	11.90 (1.66)	12.24 (1.63)	12.37 (1.67)	<0.0001
Albumin (g/L)	40.1 (3.7)	40.2 (3.7)	40.0 (3.7)	0.04	39.9 (3.8)	40.2 (3.7)	40.1 (3.8)	0.0028
C-reactive protein (mg/L) [geometric mean (approximate SE)]	3.1 (0.1)	2.9 (0.1)	3.1 (0.1)	0.09	3.3 (0.1)	2.9 (0.1)	3.0 (0.1)	0.0004
Treatment allocation								
Randomized to simvastatin plus ezetimibe	50%	51%	50%	0.52	50%	50%	50%	0.91

Mean (SD) or % shown, all characteristics adjusted for age, sex and ethnicity, with the exception of ethnicity. ACE=angiotensin-converting enzyme. ARB=angiotensin-II receptor blocker. LDL=low-density lipoprotein. HDL=high-density lipoprotein. *P value for test of heterogeneity between SBP categories. †P value for test of heterogeneity beween DBP categories.

Table S3: Guideline recommendations for management of blood pressure in chronic kidney disease

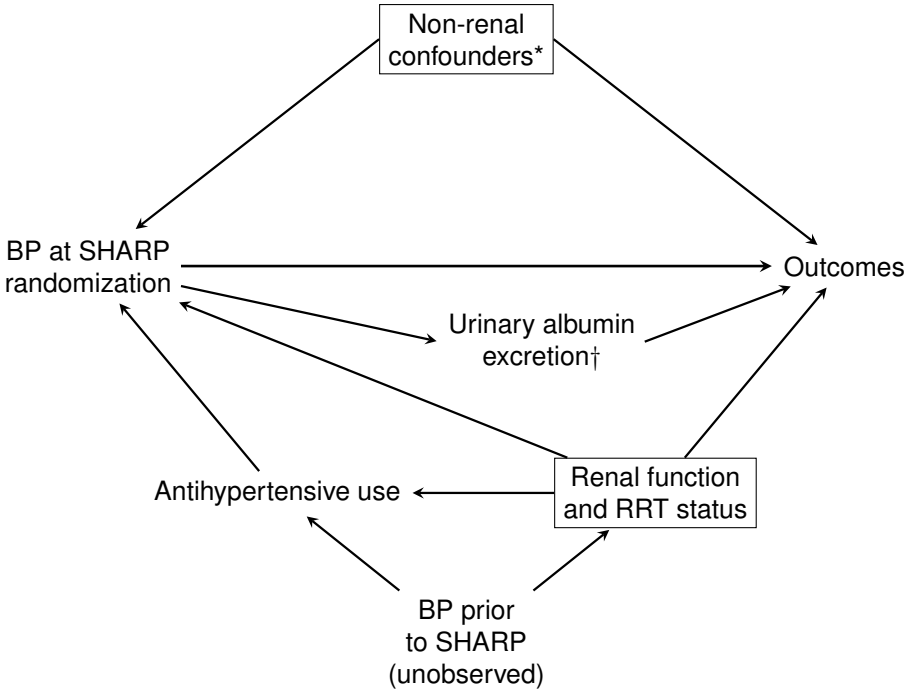
Guideline	Blood pressure target, mmHg	Target population and recommendation
Kidney Disease Improving Global Outcomes (KDIGO, 2012)[1]	≤140/90	CKD[*] or diabetes without microalbuminuria[†]
	≤130/80	CKD[*] or diabetes with micro[†]- or macroalbuminuria[‡]
Eighth Joint National Committee (JNC-8, 2014)[2]	<140/90	18-69 years and eGFR or mGFR <60 mL/min/1.73m^2 with albuminuria[§]
	<150/90	eGFR <60 mL/min/1.73m^2 and ≥70 years[‖], or CKD without albuminuria
European Society of Hypertension (ESH) and the European Society of Cardiology (ESH-ESC, 2013)[3]	<140/90	CKD[¶]
	<130/90	Overt proteinuria[#]
National Institute for Health and Clinical Excellence (NICE, 2014)[4]	<140/90	Non-diabetic CKD[*] without albuminuria[**]
	<130/80	CKD[*] with albuminuria[††]
		CKD[*] with diabetes
American College of Cardiology Foundation and the American Heart Association (ACCF/AHA, 2011)[5]	<130/80	CKD[‡‡] in elderly patients with hypertension
Canadian hypertension education program (CHEP, 2015)[6]	<140/90	Non-diabetic CKD[‡‡]
	<130/80	Diabetic CKD[‡‡]

CKD = chronic kidney disease; eGFR = estimated glomerular filtration rate; mGFR = measured glomerular filtration rate. [*] CKD defined using the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NFK KDOQI) definition as; either kidney damage (defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies) or GFR <60 mL/min/1.73m^2 for ≥3 months; [†] Microalbuminuria defined as urine albumin excretion ≥30-300 mg/d; [‡] Macroalbuminuria defined as urine albumin excretion >300 mg/d; [§] Albuminuria defined as >30 mg/g at any age and at any level of GFR; [‖] If ≥70 years, treatment should be individualised, taking into consideration factors such as frailty, comorbidities and albuminuria; [¶] CKD includes those with reduced renal function and/or the detection of elevated urinary excretion of albumin, staged according to eGFR; [#] Overt proteinuria defined as >300 mg/d; [**] Albuminuria defined as albumin:creatinine ratio ≥30 mg/mmol; [††] Albuminuria defined as albumin:creatinine ratio ≥70 mg/mmol; [‡‡] CKD defined as eGFR <60 mL/min/1.73 m^2

References for Table S3

1. Kidney Disease Improving Global Outcomes (KDIGO). KDIGO Clinical Practice Guideline for the Management of Blood Pressure in Chronic Kidney Disease. *Kidney Int.*2012;2(5).
2. James PA, Oparil S, Carter BL, et al. 2014 Evidence-Based Guideline for the Management of High Blood Pressure in Adults: Report From the Panel Members Appointed to the Eighth Joint National Committee (JNC 8). *JAMA.* 2014;311:507-520.
3. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J hypertens*. 2013;31:1281-1357.
4. National Institute for Health and Clinical Excellence (NICE): Guidance. Chronic kidney disease (partial update): Early identification and management of chronic kidney disease in adults in primary and secondary care. Clinical Guideline 182. London: National Institute for Health and Care Excellence (UK) Copyright (c) National Clinical Guideline Centre. 2014. https://www.nice.org.uk/guidance/cg182 (accessed 20th July 2015).
5. Aronow WS, Fleg JL, Pepine CJ, et al. ACCF/AHA 2011 Expert Consensus Document on Hypertension in the Elderly: A report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. *J Am Coll Cardiol*. 2011;57:2037-2114.
6. Daskalopoulou SS, Rabi DM, Zarnke KB, et al. The 2015 Canadian Hypertension Education Program Recommendations for Blood Pressure Measurement, Diagnosis, Assessment of Risk, Prevention, and Treatment of Hypertension. *Canadian J Cardiol*. 2015;31:549-568.

Figure S1: Causal diagram showing the assumed associations between baseline blood pressure, outcomes and other characteristics

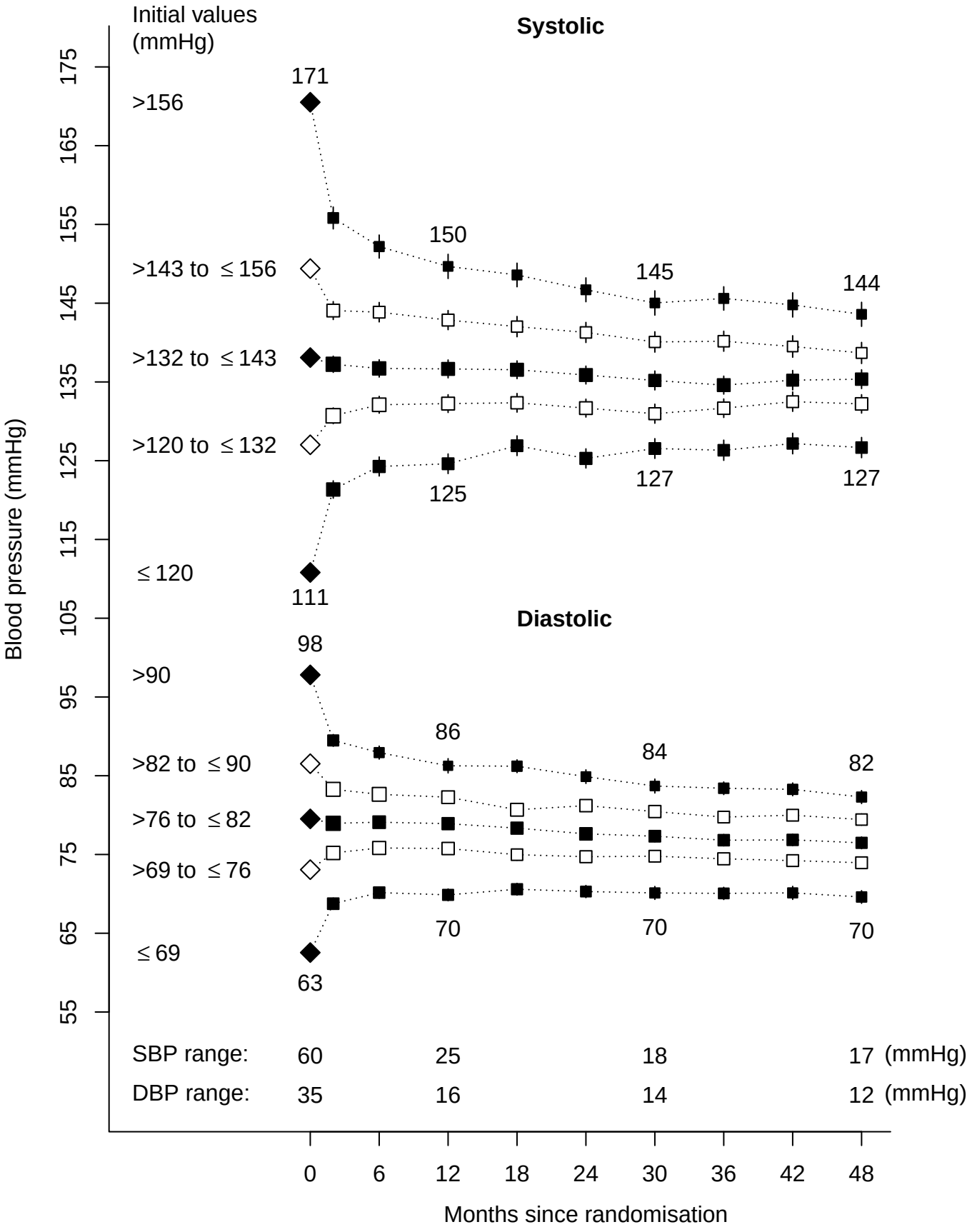


RRT=renal replacement therapy. *Age, sex, ethnicity, country, education, smoking status at screening, previous cardiovascular disease, previous diabetes mellitus and body mass index.

Analyses were adjusted for the confounders enclosed by boxes in the causal diagram. No adjustment was made for antihypertensive use as it was assumed that any effect on outcomes was mediated through its effect on blood pressure.

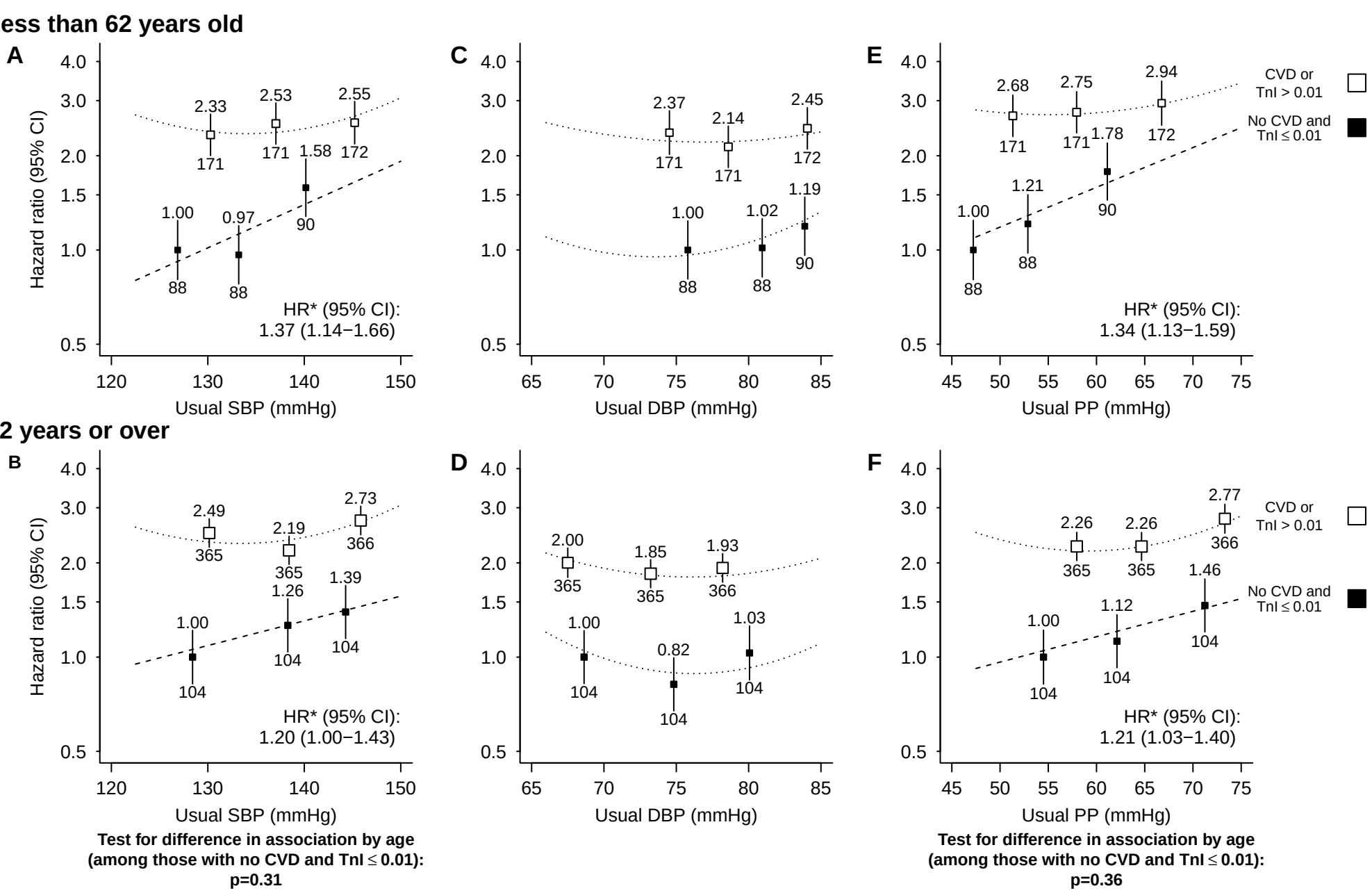
†The *a priori* assumption was that urinary albumin excretion lies on the causal pathway between blood pressure and vascular outcomes and is not a confounder, however sensitivity analyses including adjustment for urinary albumin excretion were conducted.

Figure S2: Mean blood pressure over follow−up in categories defined by quintiles of baseline measurement



SBP=systolic blood pressure. DBP=diastolic blood pressure. Excludes 4161 participants with missing BP values at any of the follow−up visits.

Figure S3: Association between systolic blood pressure, diastolic blood pressure and pulse pressure and cardiovascular events, subdivided by evidence of previous cardiovascular disease, for those less than 62 years old and 62 years or over

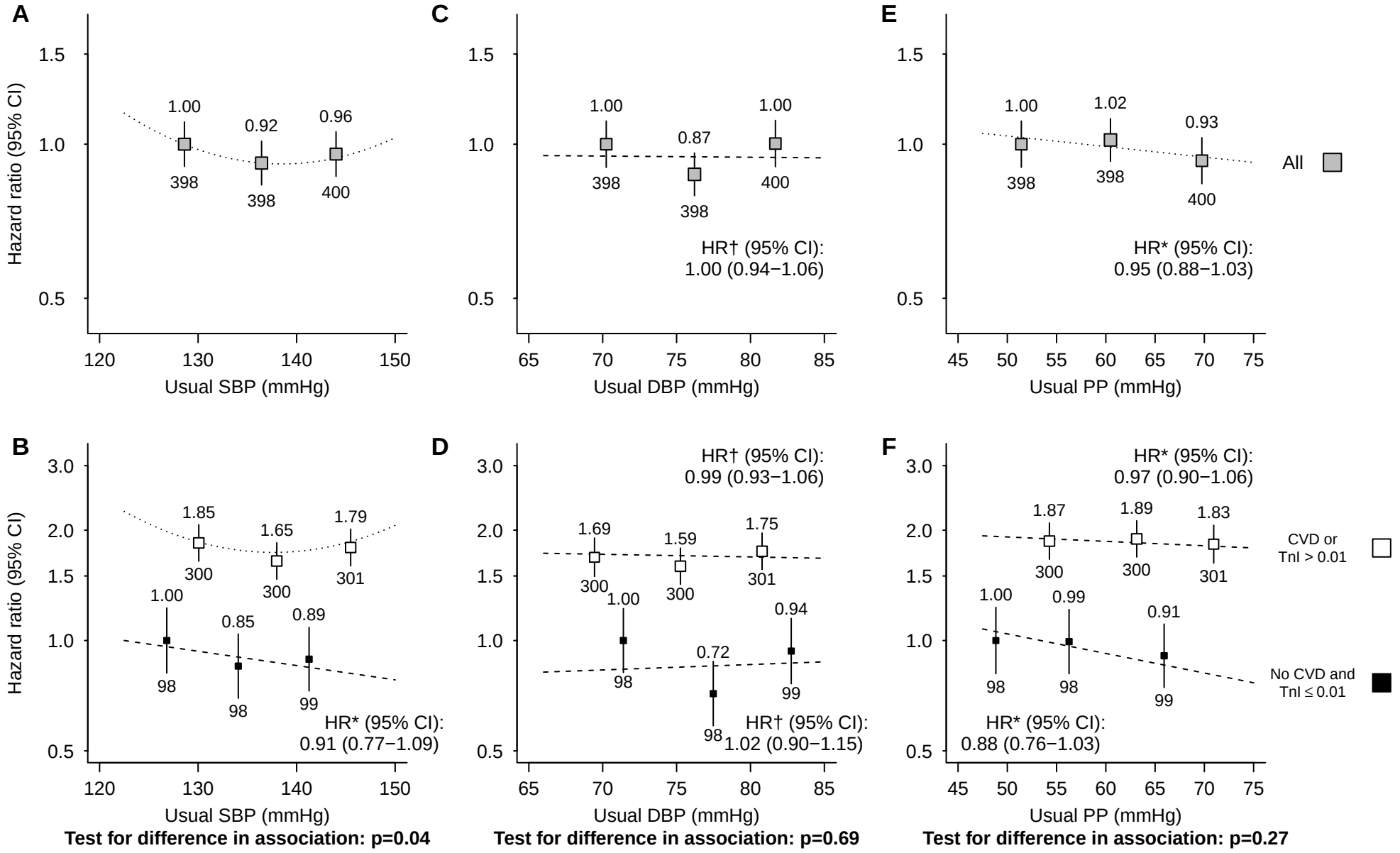


Test for difference in association by age (among those with no CVD and TnI ≤ 0.01): p=0.31

Test for difference in association by age (among those with no CVD and TnI ≤ 0.01): p=0.36

SBP=systolic blood pressure. DBP=diastolic blood pressure. PP=pulse pressure. CVD=self−reported history of cardiovascular disease. TnI=troponin−I (ng/mL). HR=hazard ratio. For each plot, categories of blood pressure contain similar numbers of events. Hazard ratios adjusted for age, sex, ethnicity, country, education, smoking status, previous diabetes mellitus, renal replacement therapy status, eGFR, body−mass index and treatment allocation are quoted (above squares) with numbers of events (below). Exclusions as per Table. *Hazard ratios per 10 mmHg higher usual SBP/PP are presented for associations where there is no evidence of deviation from a log−linear relationship.

Figure S4: Association between (A) systolic blood pressure, (C) diastolic blood pressure and (E) pulse pressure and non−vascular mortality overall, and association between (B) systolic blood pressure, (E) diastolic blood pressure and (F) pulse pressure and non−vascular mortality subdivided by evidence of previous cardiovascular disease



SBP=systolic blood pressure. DBP=diastolic blood pressure. PP=pulse pressure. HR=hazard ratio. CVD=self−reported history of cardiovascular disease. TnI=troponin−I (ng/mL). For each plot, categories of blood pressure contain similar numbers of events. Hazard ratios adjusted for age, sex, ethnicity, country, education, smoking status, previous cardiovascular disease (panels A, C and E only), previous diabetes mellitus, renal replacement therapy status, eGFR, body−mass index and treatment allocation are quoted (above squares) with numbers of events (below). Exclusions as per Table. *Hazard ratios per 10 mmHg higher usual SBP/PP and †hazard ratios per 5 mmHg higher usual DBP are presented for associations where there is no evidence of deviation from a log−linear relationship.